THE UNIVERSITY OF ALBERTA

THE ALKALOIDS OF LYCOPODIUM CERNUUM AND LYCOPODIUM ALOPECUROIDES

BY

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LICENCIADO

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

THE ALKALOIDS OF LYCOPODIUM CERNUUM
AND LYCOPODIUM ALOPECUROIDES

submitted by Serafin Valverde Lopez in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The alkaloids of Lycopodium cernuum L. have been reexamined. The structure and stereochemistry of lycocernuine, one of the main components, has been determined. It has been noted that this structure can be derived biogenetically from two polyketooctanoic acid chains as in the case of other Lycopodium alkaloids.

An interpretation of the main fragmentation modes encountered in the mass spectra of Lycocernuine and various of its derivatives is given.

The isolation and characterization of three new alkaloids from Lycopodium alopecuroides L. is reported.

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CONTENTS

INTRODUCTION

The genus Lycopodium holds an interesting place in both the botanical and the chemical fields. Although this introduction is concerned mainly with the chemistry of the genus Lycopodium, a brief discussion of the place of these plants in the plant kingdom will be given first.

In one widely used system of classification[1], the plant kingdom is divided into four divisions, Thallophyta, Bryophyta, Pteridophyta and Spermatophyta. The first two divisions are the non-vascular plants, rootless plants without a vein system, while the last two are the vascular plants, plants with roots, stems, leaves, and containing a vein system. The thallophytes include algae, fungi, bacteria and lichens, the latter formed by a union of a fungus with an alga. The bryophytes include the mosses, liverworts and hornworts. In place of roots these plants possess hairlike filaments, called rhizoids, with which they adhere to the ground.

The largest and most important group of plants are the spermatophytes or seed plants. They include the evergreen and deciduous trees and shrubs, herbs, grasses, and other flowering plants.

The genus Lycopodium belongs to the division Pteridophyta, the smallest division of the plant kingdom. The pteridophytes are the non-flowering vascular plants that reproduce by means of spores rather than seeds.

Within this division we find four classes, Equisetinae, Lycopodiinae, Isoetinae and Filicinae. The class Lycopodiinae has been subdivided into two different orders, Lycopodiales (club mosses) and Sellaginellales (spike mosses) according to the homosporous or heterosporous nature of their reproduction.

As has been mentioned above, the pteridophytes reproduce by spores. These spores are formed in receptacles called sporangium. The sporangia may contain from a few to hundreds of spores. All the spores (four) contained in each sporangium of a plant of the order Lycopodiales are alike morphologically and physiologically, hence the term homosporous. Plants of the order Sellaginellales will have two different kinds of spores (male and female) formed in separated sporangia.

Only one family, Lycopodiaceae, belongs to the order Lycopodiales. This family is subdivided in two genera. The genus Phylloglossum has only one species supposedly restricted to Australia, New Zealand and Tasmania. The other genus, Lycopodium, has more than two hundred species.

The club mosses (so called because of their moss-like leaves and club-shaped cones) were extremely abundant more than three hundred million years ago. Fossil records of their ancestors are very common in Carboniferus rocks. During this period hundreds of species of club mosses were branched trees up to several feet in diameter and a hundred or more feet in height. Together with the giant Horsetail trees and the extinct Lepidodendrous and Sigillarias they made ferneries into vast forest jungles. The beds they laid down, primarily by their tiny spores, resulted in the geological seams from which we mine coal and obtain gas.

The Lycopodiales spores are minute and uniform in size. When the sporangium opens, the spores are widely dispersed by the smallest current of air. Under suitable conditions, i. e. when they come in contact with moisture, they germinate and produce a gametophyte provided with both male (antheridia) and female (archegonia) organs. The gametophytes are very minute, rarely more than a few millimeters in length.

In Selaginella, with two kind of spores, the gametophytes that grow on germination of these spores, bear either archegonia or antheridia, but not both.

It is believed the spore takes seven or more years to develop the gametophyte. A fertilized egg of the gametophyte gives rise to the plant proper that in turn will produce spores. This alternation of sexual and asexual periods to complete the life cycle of the plant is characteristic of the Pteridophytes. Ten or more years are required before the gametophyte develops the new young plant above the ground, making a life cycle of almost twenty years from spore to gametophyte and to the plant again.

Unlike both genera* of the family Lycopodiaceae, the rest of the ferns and their allies do not seem to be alkaloid bearing plants except for some isolated instances. One alkaloid, equisetine or palustrine, has been isolated from Equisetum palustre. A few other species of the genus Equisetum, namely, E. arvense, E. telmateia and E. Sylvaticum, have been found to produce nicotine.[2]

The structure of palustrine has been determined.[3]

Since Bödeker in 1881 [4] drew attention to the fact that Lycopodium complanatum L. contained an alkaloid which he called lycopodine[5] more than fifteen different Lycopodium species have been examined with various degrees of thoroughness. Even if most of the emphasis has been placed on

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* Plants of the genus Phylloglossum (only one species) also contain alkaloids. Their structures do not seem to correspond with those of known Lycopodium alkaloids. D. N. Nkunika. Private communication.

the study of the basic material, the isolation of other natural products, in particular terpenes, has also been reported from Lycopodium clavatum[6], and Lycopodium serratum[7].

The physiological activity of individual alkaloids and that of crude mixtures has been investigated; thus annotinine and lycopodine (see below) cause contraction of isolated uterus of rat, rabbit and guinea pigs according to Marrier and Bernard[8]. The same authors also observed anti-pyretic action of both alkaloids and marked myosis in rabbits caused by annotinine. Lycopodine has also been found to possess the ability of inhibiting the necrotic action caused by tobacco mosaic virus in Nicotiana glutinosa and Datura stramonium[9]. Extracts from Lycopodium selago have been used in the treatment of chronic alcoholism, nicotinism and psoriasis[10].

With two exceptions, all of the Lycopodium species examined to date have been found to contain lycopodine (I)[11,12] or alkaloids with the same carbon skeleton as lycopodine. The two exceptions are L. saururus and L. cernuum.

The structure of sauroxine, the major alkaloid of L. saururus, which has recently been elucidated[13] is closely related to that of α-obscurine (see below).

The elucidation of the structure of the alkaloids of L. cernuum*, which represent a major departure from the commonly encountered Lycopodium

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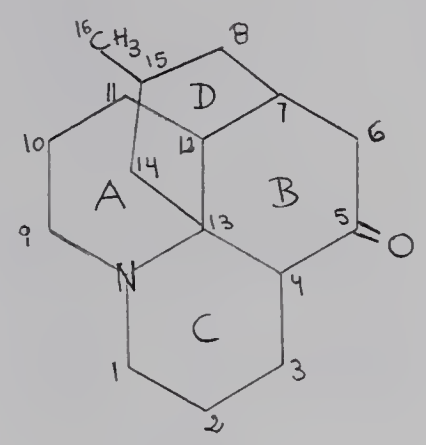
* Lycopodium cernuum grows in tropical and subtropical climates. Its presence has been reported in Jamaica, Dominica, Southern China, and South Africa[33]. Plants have been collected in Mexico and Venezuela.

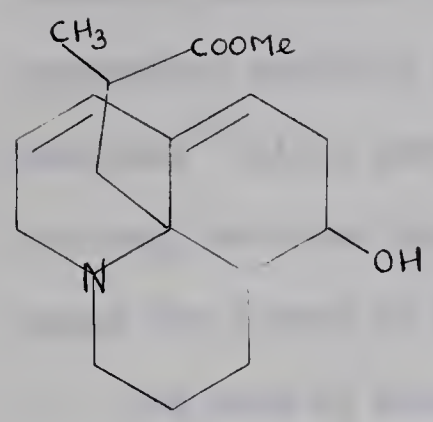
alkaloids, will be described in this thesis.



I

Alkaloids which contain the lycopodine (I) skeleton include clavolonine (8-hydroxylycopodine)[14], flabelliformine (4-hydroxylycopodine)[15] lycodoline (12-hydroxylycopodine)[16], lycoclavine, L-20 (epimeric 6-hydroxylycopodines) [17,18]; as well as others containing the basic skeleton of lycopodine with addition of further unsaturation ($\Delta^{11,12}$) such as acrifoline[19] and lycofoline[20].

Several alkaloids which do not contain the lycopodine skeleton, but which are obviously patterned along the same general lines, are known. Annotinine[21], the first alkaloid of the group to have its structure elucidated has structure (VII). Compounds such as lyconnotine (II)[22] are still closely related to lycopodine (lyconnotine can be formally derived by an oxidative cleavage of ring D, from a precursor such as acrifoline). Slightly rearranged skeletons are encountered in the obscurines (α- and β- obscurine)(III, IV)[23,75],



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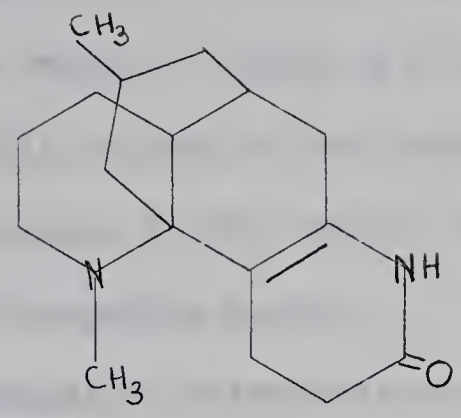

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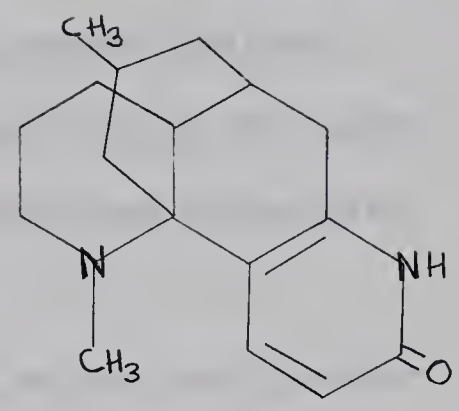

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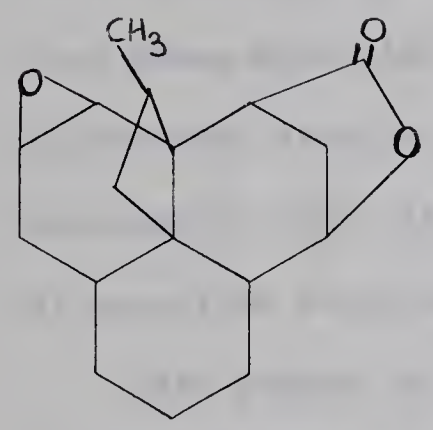

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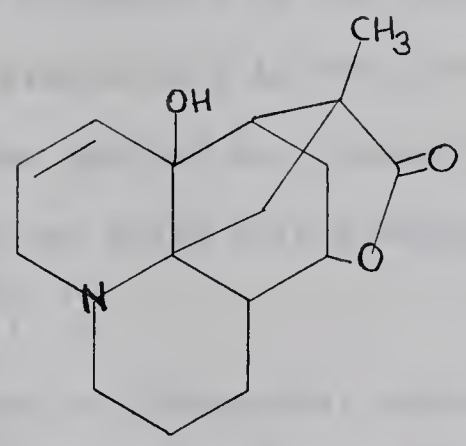

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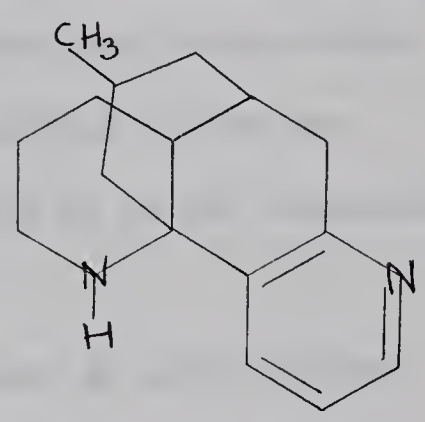

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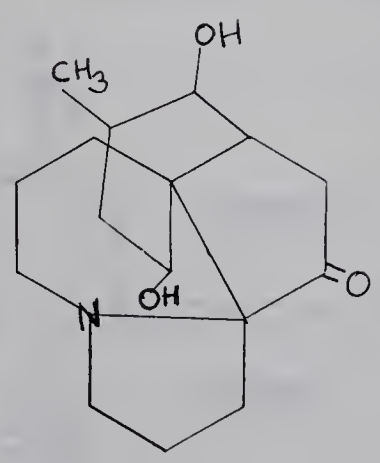

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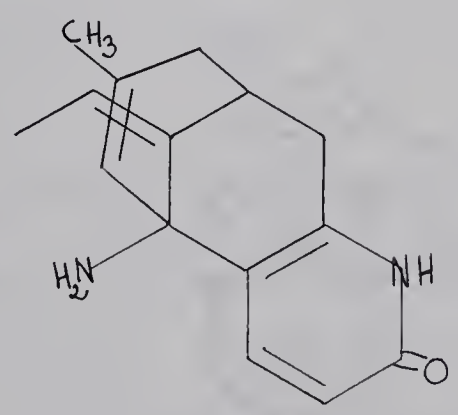


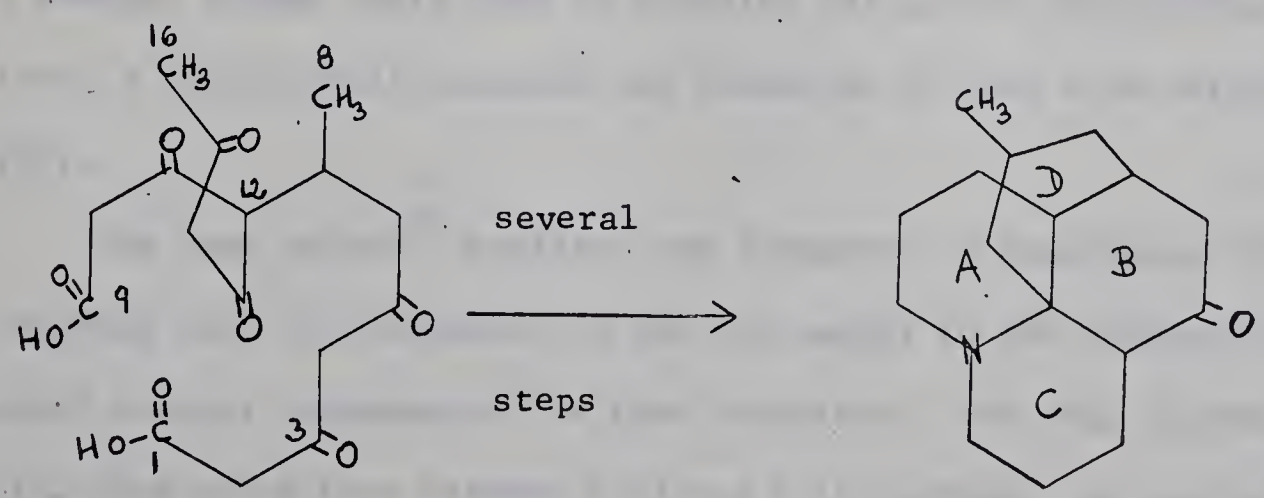
IX

lycodine[24] (V) and annotine[25] (VI). The structure of serratinine (VIII), recently published[26], exemplifies a unique type of rearrangement of the lycopodine skeleton (see below). Finally a fifteen carbon alkaloid, selagine[27] (IX), apparently related to the obscurines (C-9 is the carbon missing) adds one more example to the variety of structures encountered among the plants of the Lycopodium genus.

The mode of biosynthesis of these alkaloids is as yet poorly understood, although a plausible biogenetic scheme has been proposed[28]. The Conroy proposal[28] for the biogenesis of the lycopodium alkaloids suggests that these alkaloids are synthesized in the plant from two eight-carbon polyacetate straight chains derived in a manner analogous to the one believed to take place in the fatty acid biosynthesis or in the formation of macrolide antibiotics[29].

This scheme as applied to lycopodine, begins with an aldol condensation between the C-7 carbonyl and the C-12 methylene, followed by dehydration, (Scheme A)

Scheme A

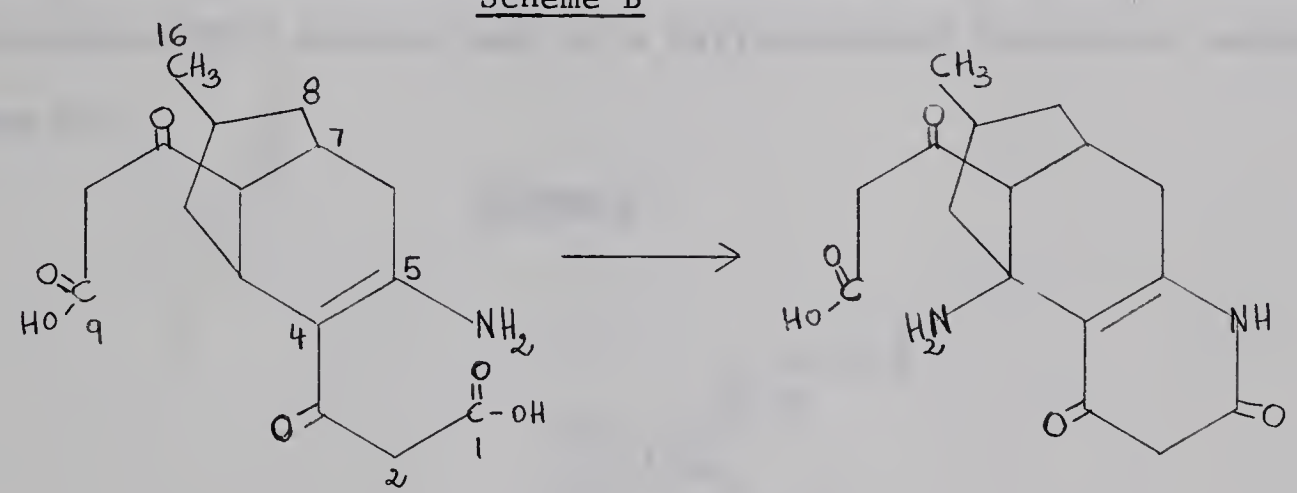


I

A second aldol between C-8 and C-15 would close ring D. After reduction of the double bond a Mannich condensation with a molecule of ammonia would join C-4 and C-13. Then both lactam rings (A and C) are completed and the oxidation level of the carbons is adjusted.

Basically the same sequence would explain the formation of the obscurines; in this case the Mannich condensation is followed by amination of C-5 and the formation of the lactam ring C, (Scheme B).

Scheme B



N-Methylation followed by lactam formation (ring A) and adjustment to the proper oxidation level would afford the obscurines (III and IV), a similar scheme could lead to lycodine (V) and if decarboxylation (C-9, a β-keto acid) precedes the formation of ring A we obtain selagine (IX).

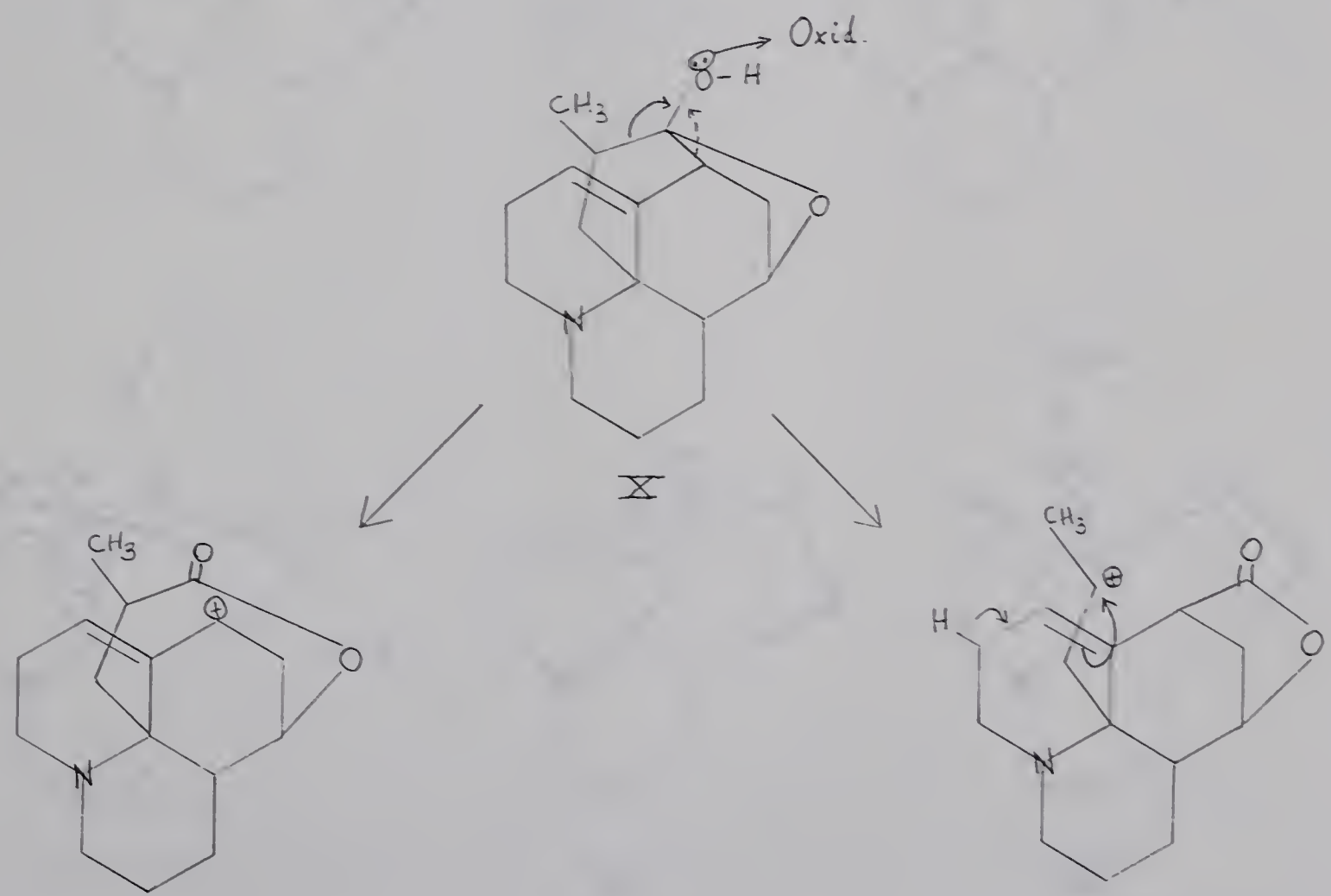
The same author[28] explains the formation of annotinine (VII) assuming that the oxidation of the C-8 methyl to the carboxylic stage would prevent condensation in that direction. The ring closure reaction will take place then between C-12 and C-15 instead. As will be seen this biogenetic scheme was of great value in determining the structures of the L. cernuum alkaloids.

The widespread presence of the lycopodine skeleton among the

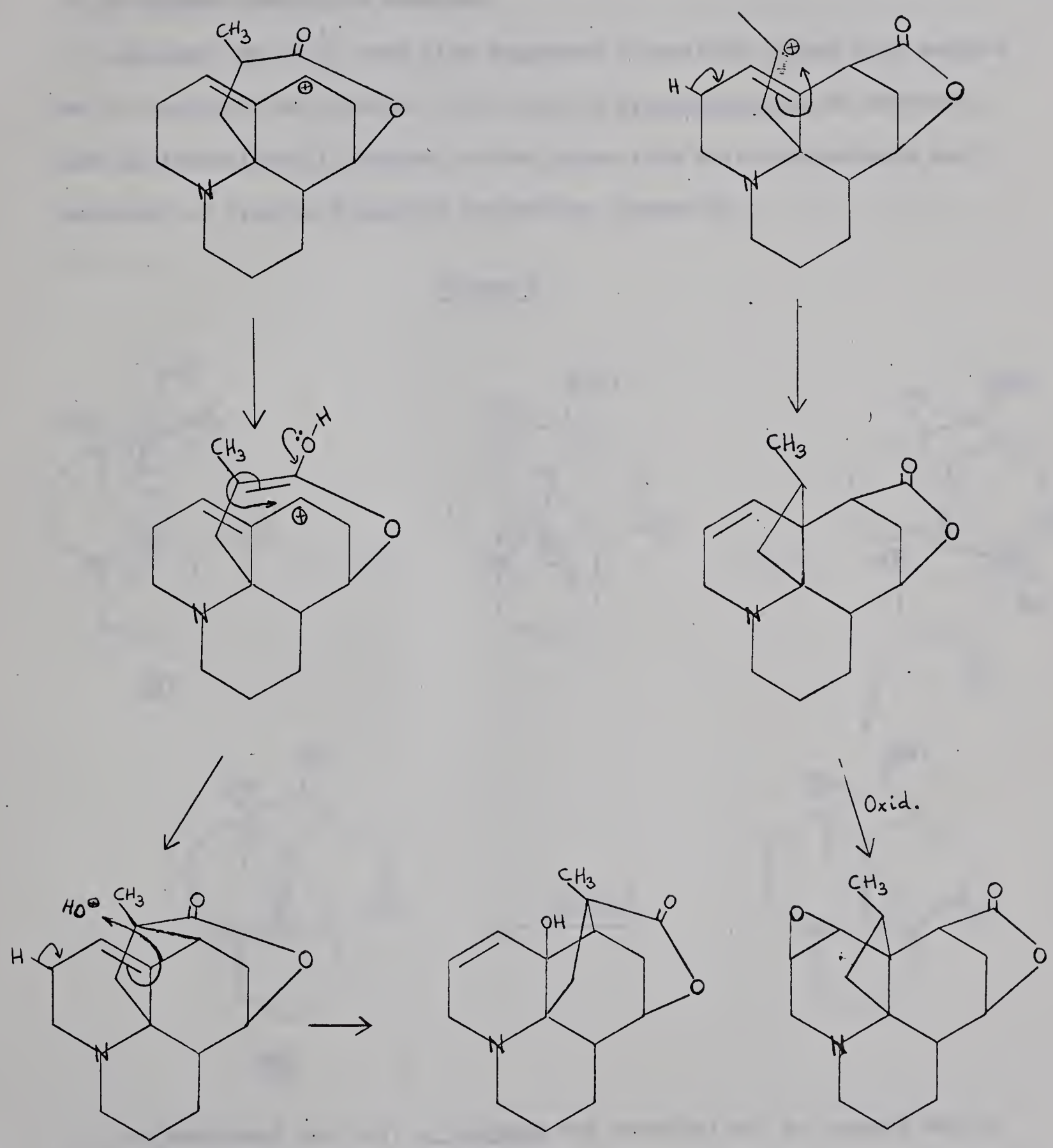
Lycopodium alkaloids seems to indicate a special place for this skeleton in any proposal for a comprehensive biogenetic scheme aplicable to these alkaloids. This view is certainly supported by the isolation of a host of alkaloids (partially listed earlier) all containing a functionalized lycopodine skeleton.

Wiesner has pointed out[30] that, possibly, the Lycopodium plants can accomplish the interconversion of lycopodine type of alkaloids such as annofoline[31] (or acrifoline[19] (X)) into others such as annotine (VI) or annotinine (VII) without need of a differenciated biogenetic pathway (Scheme C).

Scheme C



Scheme C

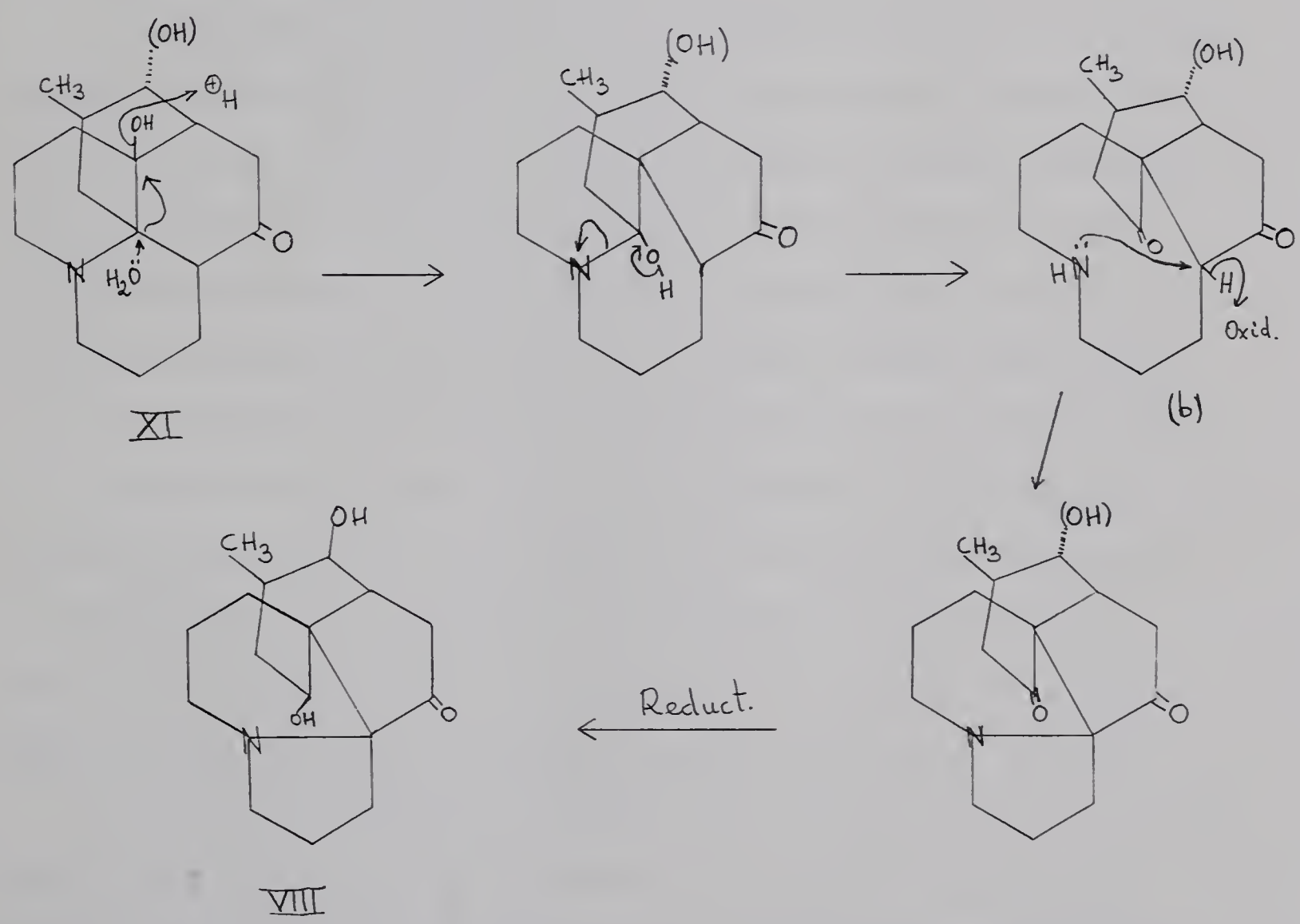


VI VII

Lyconnotine[22] (II), could also be derived from the first intermediate of the scheme leading to annotine.

Inubushi et al.[32] have also suggested a possible scheme that enables one to correlate serratinine (VIII) with a lycopodine-type of alkaloid such as lycodoline XI (present in the plant from which serratinine was isolated) or from an 8-hydroxy lycodoline (Scheme D).

Scheme D



As mentioned earlier, L. cernuum was reported not to contain any of the more common Lycopodium alkaloids[84], but to contain two alkaloids unique to this species. This, coupled with the fact that an examination

of the spectroscopic properties of one of these alkaloids* indicated that it did not fit into the pattern of the other alkaloids, led us to examine the alkaloids of this species in detail.

The fact that the alkaloids of L. alopecuroides had not previously been examined, coupled with the circumstance that a source of the crude alkaloids was available to us, prompted us to initiate work on this species.

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* The initial spectra were determined on a sample of cernuine kindly furnished by R. H. F. Manske.

DISCUSSION AND RESULTS

In their early work on the alkaloids from Lycopodium cernuum L., Marion and Manske[74] reported the isolation of three different compounds. One of them, L-32 or cernuine, was assigned the molecular formula $C_{16}H_{26}ON_2$ on the basis of the analysis of both the free base, m.p. 106°, and the perchlorate, m.p. 110°. They also reported the presence of nicotine plus a third base, (L-33, m.p. 218°) which was present only as a minor component in the plants studied by these authors.

In the course of our studies with the alkaloids produced by Lycopodium cernuum we have had occasion to investigate crude alkaloid extracts from plants collected in Venezuela, Florida, and Mexico. No major differences were encountered in the alkaloidal content from the different sources, although some extracts contained more basic material than others. Both alkaloids L-32 and L-33 were found to be present in almost equal amounts and they constituted the bulk of the alkaloidal contents of this species.

Besides L-32 and L-33, other bases are present in small amounts. Some attention has been given in our laboratory* to the study of those minor components but, so far, no positive identification has been achieved, except for one other alkaloid which proved to be identical with dihydro-deoxycernuine, the product obtained by hydride reduction of cernuine.

To obtain the crude alkaloid mixture the following procedure was used: the dried plant material was percolated with methanol. The methanolic extract was evaporated to dryness and the residue was digested overnight with warm, aqueous tartaric acid (3%). This aqueous solution was

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* J. K. Jenkins. Private communication.

depleted of neutral material by extracting with chloroform, then basified and extracted with chloroform to yield the crude mixture of bases. Eleven kilograms of dried plant, collected in Venezuela, gave 12.7 g of crude bases*. In our hands eight kilograms of dried plant, collected in Mexico, afforded 5 g of crude alkaloids.

When these crude alkaloids were chromatographed over alumina, both L-32 and L-33 were isolated. In a typical experiment (crude alkaloid, 1 g; alumina, 30 g) elution with ether yielded cernuine (L-32, 150 mg), while elution with chloroform furnished L-33 (220 mg).

The relative ratio of L-32 to L-33 varied from one crude extract to the other, cernuine becoming the major component occasionally, but at no time have we encountered an extract in which L-33 was present in minor or trace amounts. The identity of those alkaloids was established by direct comparison with samples supplied by R. H. F. Manske.

The physical and chemical properties of alkaloid L-33, which led to the establishment of its structural formula and to the determination of its stereochemistry will now be described. When the clarity of the discussion so requires our findings will be interrelated with the results obtained in our laboratory with alkaloid L-32**. As we shall see later these alkaloids (L-32 and L-33) are closely related.

After recrystallization from acetone-ether, L-33, which we have named lycocernuine, (see Fig. 1), is a colorless, crystalline compound, m.p. 230°***. From analytical and mass spectrometric data the molecular

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* R. H. Burnell. Private communication.

** J. K. Jenkins. Private communications.

*** L-33, or lycocernuine, has been obtained in two crystalline isomorphic forms with m.p.'s 230° and 210-212°.

formula $C_{16}H_{26}O_2N_2$ was assigned to it. Other spectroscopic data obtained from this alkaloid proved to be very informative. The infrared spectrum of lycocernuine (carbon tetrachloride solution) shows amide absorption at 1640 cm^{-1} and a moderately intense band at 1410 cm^{-1}. Both L-32 and L-33 display these bands in their infrared spectra. The band at 1410 cm^{-1} disappears when the amide group is reduced and is displaced to lower frequency when the compound is treated with sodium methoxide in methanol-O-d (during this treatment two deuterium atoms are incorporated, as revealed by mass spectrometry). This suggested the presence of the grouping,

$$- N - \underset{\underset{O}{\|}}{C} - CH_2 -$$

in the molecule. In addition there is a band at 3620 cm^{-1} indicative of a non H-bonded hydroxyl group. This accounts for the second oxygen atom present in the lycocernuine molecule.

As has just been pointed out, one of the nitrogens forms part of an amide (or lactam) group. Accordingly lycocernuine titrates as monoacidic base [pK'_a value (50% CH_3OH): 6.4]. That the second nitrogen is tertiary may be inferred from the fact that lycocernuine forms a C_{17} methiodide. Lack of NH absorption indicates that the amide (or lactam) is tertiary.

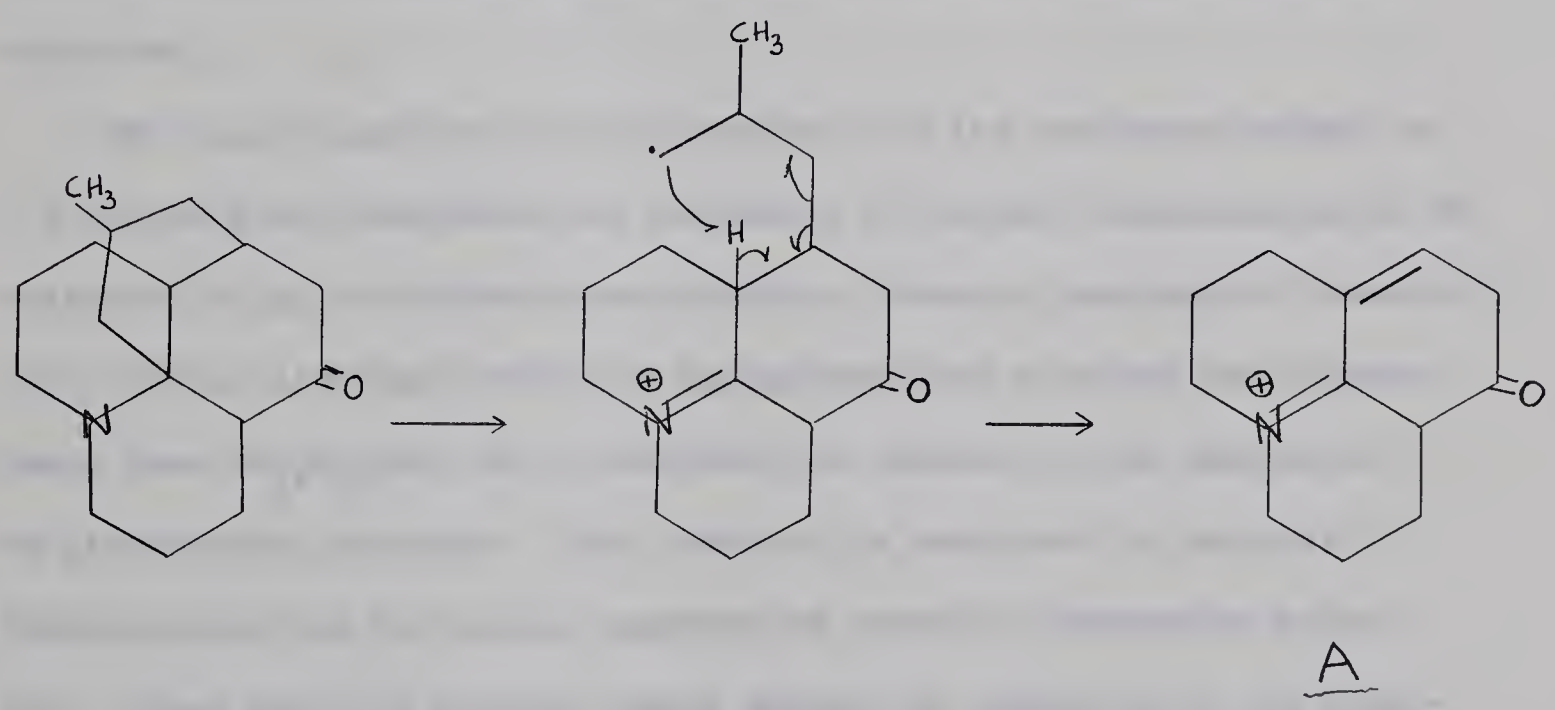
Cernuine also shows infrared absorption at 1640 cm^{-1}, lacks NH absorption, titrates as a monoacidic base [pK'_a (50% CH_3OH): 6.3] and forms a C_{17} methiodide. Lycocernuine presents the distinctive characteristic of possessing a hydroxyl group that is not present in cernuine.

The nuclear magnetic resonance spectrum of L-33 shows a one proton

signal at τ 4.55 and a three proton doublet at τ 9.15 (J: 6.5 cps). Analogous signals are present in L-32. The doublet at τ 9.15 can readily be assigned to a methyl group attached to a methine carbon. In L-33, but not in L-32, we find a poorly resolved multiplet at τ 6.25 possibly associated with the OH group of L-33.

The preliminary analysis of the mass spectrum of lycocernuine, apart from showing the molecular ion ($M^{\oplus}$, m/e 278), helped to establish some guidelines for the ensuing work on lycocernuine.

A rationale for the mass spectra of lycopodium alkaloids has been offered[34]. The base peak of alkaloids with a lycopodine type of skeleton can be interpreted as follows:



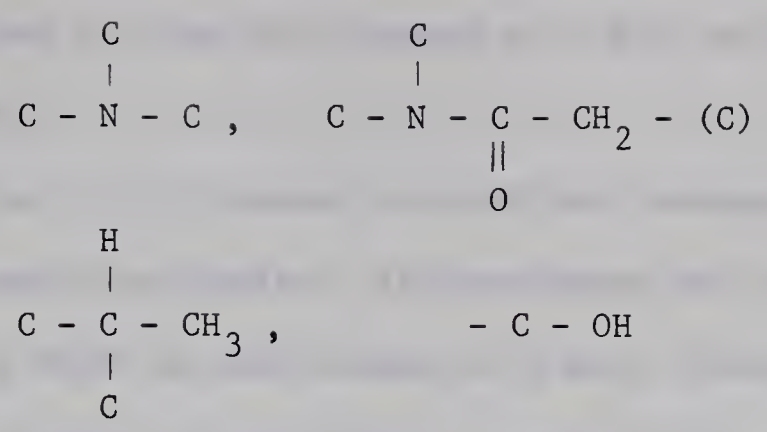
Loss of the bridge accounts for the main fragmentation path. Alkaloids of the lycopodine type with no substitution on the bridge carbon atoms thus exhibit a strong peak at $M^{\oplus}$-57 corresponding to ions of type A. Those with a keto- or hydroxyl-function at the bridge carbons show intense peaks at $M^{\oplus}$-71 or $M^{\oplus}$-73. These generalizations apply also to alkaloids with two nitrogens such as the obscurines.

The main fragments in the mass spectrum of cernuine are found at $M^{\oplus}$-29 (100), M-42 (74), M-43 (48) and do not correspond to those of lycopodine-type alkaloids. On the other hand, the mass spectrum of lycocernuine also displays prominent fragments at M-29 (23), M-45 (12), M-58 (60) and M-59 (100). Furthermore the pattern of both mass spectra below m/e 200 is almost identical.

This data made it appear highly unlikely that those alkaloids could have a lycopodine-type skeleton or even be closely related to known lycopodium alkaloids containing two nitrogens such as lycodine (V) or the obscurines (III), (IV). At the same time the similarities in the spectra strongly suggested that L-32 and L-33 have identical carbon skeletons.

The n.m.r. spectrum of lycocernuine with its one proton signal at τ 4.55 could be interpreted as indicative of further unsaturation in the molecule (e. g. a trisubstituted olefin). However treatment of base L-33 with lithium aluminum hydride in tetrahydrofuran afforded the dihydrodeoxy base ($C_{16}H_{28}ON_2$) which exhibited no carbonyl or NH absorption in its infrared spectrum. This compound is resistant to catalytic hydrogenation and its n.m.r. spectrum is devoid of absorption below τ 6.0. Since the only obvious change during the reduction is the transformation of the amide or lactam carbonyl to methylene, it appeared very unlikely that the τ 4.55 signal is due to an olefinic proton. The upfield shift undergone by this proton on reduction of the amide group suggests it is associated with the carbonyl group of the alkaloid.

It is convenient at this point to summarize the functional groups and partial structures so far described:



To account for the molecular formula $C_{16}H_{26}O_2N_2$, considering that no further unsaturation has been detected, the molecule must be tetracyclic. Since no N - CH_3 or N-alkyl is detected both nitrogens must form part of a ring system. Therefore the partial structure - N - C(=O) - defines a lactam grouping.

From the outset, we believed that it would be in the best interests of this research if we were able to correlate the results obtained with L-33 with those available from cernuine, especially since the paucity of alkaloidal material cast some doubts on whether or not it would be possible to carry out a thorough degradative analysis. Our early efforts were therefore directed toward establishing a correlation between the two alkaloids.

Base L-33 is easily acetylated with acetic anhydride-pyridine at room temperature. The basic acetyl derivative of L-33, $C_{18}H_{28}O_3N_2$, molecular wt. 320, failed to crystallize and was purified by molecular distillation. Its infrared spectrum (carbon tetrachloride solution) shows no absorption in the OH and NH regions, confirming again the tertiary nature of both nitrogens. In addition to the lactam absorption (1640 cm^{-1}), two new bands at 1730 and 1230 cm^{-1} confirmed the presence of an O-acetyl group in the derivative.

The nuclear magnetic resonance spectrum of O-acetyl lycocernuine

(See Fig. 2) shows the low field proton at τ 4.45 as a quartet (splittings 11.5 and 2.8 cps).

The signal at τ 6.25 present in L-33 has, however, shifted down to τ 5.06 in the acetyl derivative. In both cases this signal is a complex multiplet with a width at half-height of 5 cps. Since this signal integrated for one proton, the hydroxyl group in L-33 must be secondary. The n.m.r. data (half-height width) suggests an axial conformation for the hydroxyl group[35].

In our first attempt to correlate L-33 with cernuine we tried to convert the alkaloid in its dehydro derivative by oxidation. Since we appeared to be dealing with a secondary alcohol with axial conformation no difficulty was anticipated[36,37]. Assuming that L-33 was simply a hydroxylated derivative of cernuine (L-32) oxidation of L-33 followed by Wolff-Kishner reduction might provide the desired correlation.

When L-33 was oxidized with chromium trioxide in glacial acetic acid a gummy product was obtained which, after purification by chromatography, yielded a compound with no hydroxyl absorption in its infrared spectrum and a new carbonyl band at 1705 cm^{-1}. However the low yield obtained by this method made advisable the search for other procedures.

Oxidation with dicyclohexylcarbodiimide-dimethyl sulfoxide[38] failed, most of the starting material being recovered unchanged. The best results (50 - 60% yield) were obtained using Jones' reagent (chromic acid in acetone)[39].

The dehydroderivative of L-33 is a solid, m.p. 161-4°, easily purified by sublimation. Its molecular weight (276) and analytical data correspond to the molecular formula $C_{16}H_{24}O_2N_2$. Strong bands in its

infrared spectrum at 1705 and 1635 cm^{-1} could be assigned to a six-membered ketone and the lactam respectively. This L-33 derivative exhibited a surprising lack of basicity as evidenced by its inflection-free pK curve obtained when it is titrated with 1N sulfuric acid in 80% methyl cellosolve and by its ease of extraction from acid solution.

The weakly basic character suggested an α-aminoketone system, which is also suggested by the $n \rightarrow \pi^*$ absorption at 318 mμ (ε = 64) in the ultraviolet* and by the single Cotton effect[78] (extrema at 339 and 292 mμ) above 250 mμ in its optical rotatory dispersion curve. This latter data is sufficient to rule out the possibility of a five membered lactam grouping, which is consistent only with the lack of basicity and the frequency (1705 cm^{-1}) of the infrared absorption of the newly introduced carbonyl group. Additional chemical evidence supports this view.

The dehydro derivative of L-33 is easily converted into the starting material by treatment with sodium borohydride in methanol. The absence of carbinolamine properties in L-33 (as well as in the epimeric compound also obtained as a minor component in the sodium borohydride reduction) implies that the hydroxyl group is not attached to the carbon alpha to the basic nitrogen.

The next step in our correlation of L-32 and L-33 was a Wolff-Kishner reduction (Huang-Minlon modification)[40] of the dehydro derivative of L-33. A complex mixture of products was obtained. On t.l.c. a component with the same R_F as cernuine was detected but attempted separa-

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* Tropin-2-one, for example shows $\lambda_{max.}$ 314 ε = 64[48].

tion by chromatography on alumina failed to yield this component in a pure form and no positive identification could be achieved. The infrared spectrum, mass spectrum and t.l.c. behaviour, though, were remarkably similar to those of cernuine. These results obtained with a small (4 mg) and not completely pure sample, though not conclusive, did strongly indicate that our assumption that base L-33 was merely a hydroxylated derivative of cernuine was probably correct. Hereafter we refer to base L-33 as lycocernuine only.

In our search for alternatives to the Wolff-Kishner reduction, the method of Clemensen (amalgamated zinc and acidic medium) did not seem to offer very good prospects because of the numerous reports[41,42] of cleavages that take place when working with α-aminoketones. The treatment of dehydrolycocernuine with amalgamated zinc did in fact produce a complex mixture whose components (at least three) were more polar than cernuine (t.l.c.) and exhibited OH and/or NH absorption in the infrared. Reduction with zinc-dust and acetic acid[43] gave starting material as the only product.

A modification of the Wolff-Kishner reduction which has been successfully applied to α-aminoketones has been reported by Henbest[44]. Toluene is the solvent used and the basic catalyst is potassium tert-butoxide. Dehydrolycocernuine hydrazone was submitted to the conditions described by Henbest. The reaction time allowed was fifteen hours. This led to the isolation of a very complex mixture from which, by carefully executed chromatography, a few milligrams of a cernuine-like compound (t.l.c., infrared) were separated.

The modification described by Cram[45] was next employed (solvent,

dimethyl sulfoxide; basic catalyst, potassium tert-butoxide). In our hands no reduction took place and most of the hydrazone was recovered unchanged.

We also investigated a method first described by Caglioti and Grasselli[46] and which has recently been used in the steroid field[47]. The p-toluensulfonyl hydrazone of dehydrolycocernuine was prepared and reduced with sodium borohydride in methanol. Once more we managed to isolate material tentatively identified as cernuine (infrared) but the yield was very low and a crystalline sample of cernuine was not obtained. An attempt at direct conversion of lycocernuine into cernuine by treatment of the former with freshly distilled hydroiodic acid[15] gave back starting material.

Attempts at a rigorous correlation were therefore postponed until we had gained more knowledge about the chemical behaviour of lycocernuine and its derivatives.

As mentioned previously the n.m.r. spectra of both lycocernuine and cernuine show a low field proton at τ 4.55. Since this signal moves upfield (τ 6.4) in the dihydrodeoxyderivatives of both alkaloids[49], this proton must be connected with the lactam group. The chemical shift seems to be rather low for a hydrogen atom attached to a carbon alpha to the lactam nitrogen*. It was thus assumed that this particular carbon atom was alpha to both nitrogen atoms in the molecule. In other words the nitrogen atoms are beta to each other as in scheme [A]. This scheme also

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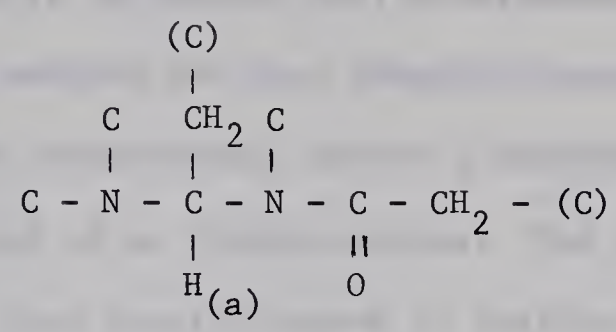
* Usual range for such protons τ 6.6 - 6.7 (Varian Associates Catalog).

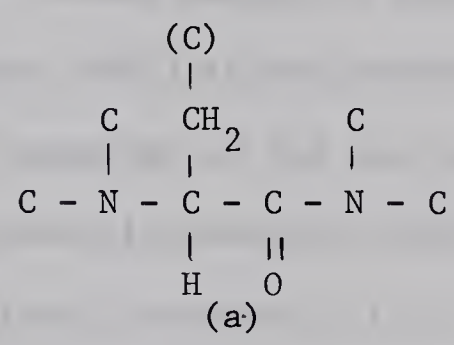
incorporates other features previously discussed.

Scheme [A]



The alternative arrangement, (Scheme [A']),

Scheme [A']



was not considered likely on the basis of the chemical shift data*; in addition the fact that the coupling pattern of proton (a) in the parent compound (a quartet) remains unchanged after the reduction with lithium aluminum hydride rules out the viability of such an arrangement. Scheme [A'] will also not account for the presence of a methylene alpha to the lactam carbonyl. The chemical shift of proton (a) in the reduced product is also in agreement with the first proposal[50]. To add support to our

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* Hydrogens such as $-N-\underset{\underset{O}{\|}}{C}-CH_2-$ are reported to absorb at τ 7.7 - 7.8, at higher field than $-\underset{\underset{O}{\|}}{C}-N-CH_2-$ (τ 6.6 - 6.7).

assignment the methiodides of both lycocernuine and cernuine[49] were prepared. These derivatives showed a down-field shift of 0.2* and 0.4 ppm, respectively, for proton (a), in agreement with [A].

Previously we pointed out that dehydrolycocernuine (obtained by Jones' oxidation of lycocernuine) showed a remarkable lack of basicity and features expected of an α-amino ketone. The preparation of anhydrolycocernuine could thus possibly serve to confirm the proposed beta relationship between the hydroxyl group and the nitrogen.

Attempts to carry out this dehydration using phenylphosphonyl dichloride in pyridine[24] caused extensive loss of the alkaloid material. Pyrolysis of lycocernuine (240°) in the presence of pyridine-impregnated alumina[51] gave starting material as the only product. Equally unsuccessful was the pyrolysis of O-acetyllycocernuine (300°); starting material was the only product identified (infrared, t.l.c.).

The reaction of lycocernuine with p-toluenesulfonyl chloride in pyridine was very slow. No p-toluenesulfonic ester was isolated but, after forty hours of reaction time, there was evidence (mass spectrum and t.l.c.) to show that the desired anhydroderivative of lycocernuine had been formed to a small extent. With this result in hand we tried next the reaction of lycocernuine with methanesulfonyl chloride in pyridine. Under these conditions lycocernuine was transformed into its mesylderivative and this on treatment with sodium methoxide in methanol readily gave the desired elimination product.

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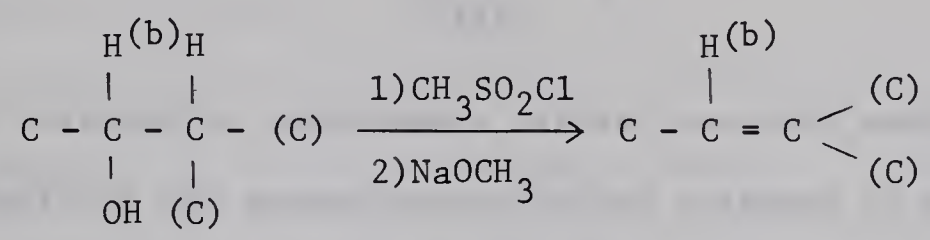
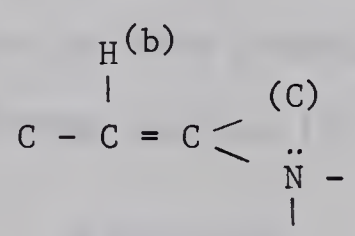
* Due to low solubility in the standard solvents, the n.m.r. of lycocernuine methiodide was measured in a mixture of heavy water and deuteroacetone.

Anhydrolycocernuine [m.p. 140 - 142°, molecular wt., 260 (mass spectrometry)], showed properties of an enamine. The n.m.r. spectrum of anhydrolycocernuine displays a one proton signal at τ 5.27. This signal was assigned to the olefinic proton (b) in the double bond just created:



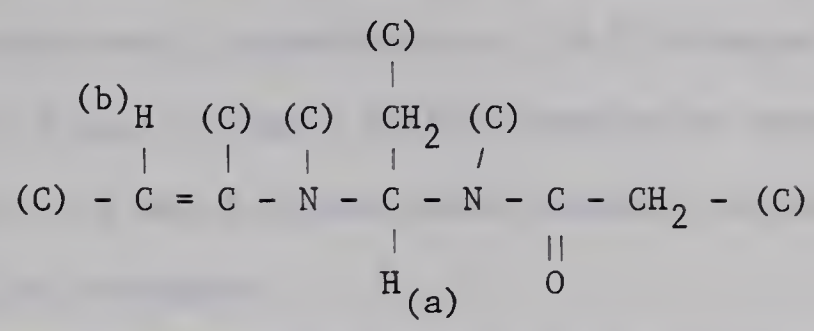
The chemical shift of (b) is higher than is expected from a normal olefinic proton (τ 4 - 4.8) but this is within the range in which enamine protons beta to the nitrogen usually appear[52]. The infrared spectrum shows a band at 1655 cm^{-1} (in addition to the lactam band at 1635 cm^{-1}) which is also indicative of the enamine system[53]. Therefore we can adjust the partial formula written above to account for the beta enamine nature of (b) as follows:



The anhydrocompound was very sensitive to acid and attempts to prepare immonium salts (the immonium salt would have been useful in confirming the enamine nature of anhydrolycocernuine since it is known[54] that the infrared band assigned to the enamine grouping (1634 - 1655 cm^{-1}) is shifted to higher wave number (1670 - 1645 cm^{-1}) on formation of the immonium salt), were unsuccessful.

If we incorporate these results into our previous scheme [A], we are now able to extend the partial structure to that shown in scheme [B].

Scheme [B]

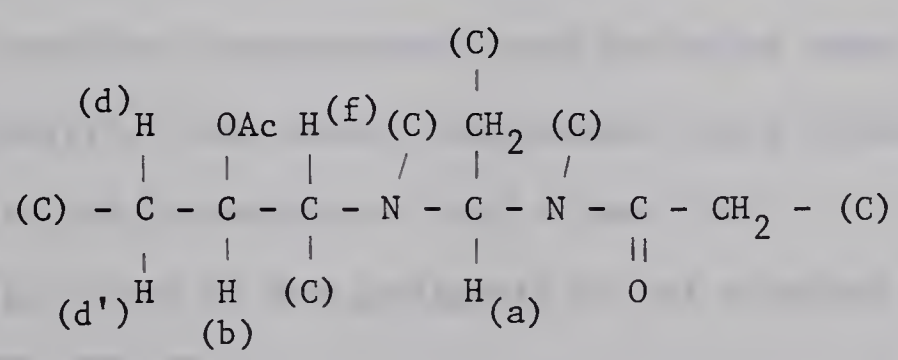


Double irradiation experiments carried out with anhydrolycocernuine greatly simplified the unresolved multiplet assigned to the enamine proton (b) and helped to further define its environment.

Simultaneous irradiation at τ 7.57 (2.4 ppm upfield from the enamine proton), caused the signal for proton (b) to collapse to a clean doublet (J = 4.5 cps). On simultaneous irradiation at τ 7.72 (2.55 ppm upfield from (b)) this proton appeared as a broad singlet (or unresolved doublet).

These results are best interpreted by assigning these chemical shifts (τ 7.57 and 7.72) to geminal allylic protons. This is consistent with similar experiments carried out with O-acetyl-lycocernuine which can be best interpreted using a scheme such as [C].

Scheme [C]



The complex multiplet corresponding to proton (b)(at τ 5.06)(see Fig. 2) present in the n.m.r. spectrum of O-acetyllycocernuine, becomes roughly a triplet (or doublet of doublets with identical coupling constants) when

we simultaneously irradiate at τ 6.8 (probably proton (f)).

On the other hand, irradiation at τ 8.13 changes the multiplet to a doublet (J = 2 cps). When a triple irradiation experiment is conducted in which both τ 6.8 and 8.13 are simultaneously irradiated, the multiplet (b) collapses to a singlet.

These results are consistent with an arrangement of atoms such as the one depicted in [C]; proton (f) being assigned a chemical shift of τ 6.8, while protons (d) and (d') (probably of very close chemical shift) are buried in the τ 8.1 region.

Also consistent with these assignments are the following facts: While in the n.m.r. spectrum of O-acetyllycocernuine there are three protons between τ 6.2 and 7.0 (assigned to protons α-to nitrogen), in anhydro-lycocernuine there are only two such protons in this area and in dehydro-lycocernuine there is a signal (roughly a quartet) at τ 6.28 which may be assigned to proton (f) (see scheme [C]).

Finally, while lycocernuine itself does not exchange any of its hydrogens for deuterium atoms (other than OH to OD) when treated with a mixture of deuterochloric and deuteroacetic acids, its keto derivative (dehydrolycocernuine) incorporates three deuterium atoms as shown by mass spectrometry*. This result compliments those obtained from the n.m.r. spectra and is consistent with scheme [C].

A chemical proof of the assignment of the relative position of the

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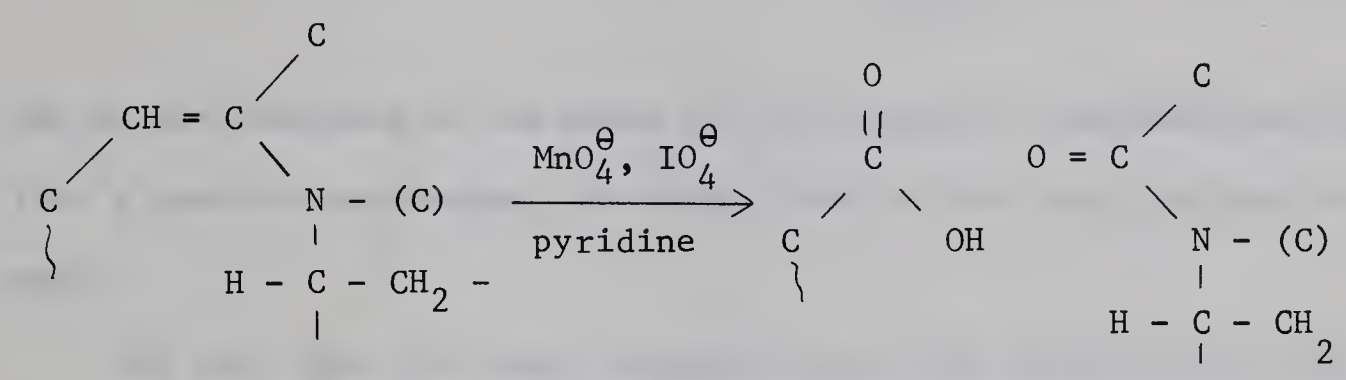
* To prevent loss of the deuterium incorporated, the deuterated ketone was reduced with sodium borohydride in deuteromethanol and the deutero-lycocernuine obtained used for mass spectrometry.

hydroxyl group in lycocernuine with regard to the basic nitrogen was obtained by two interrelated sets of experiments carried out with anhydrolycocernuine and dehydrolycocernuine.

Oxidation of anhydrolycocernuine with the Lemieux-von Rudloff reagent[55], using aqueous pyridine as the solvent, gave an acidic compound with the same number of carbon atoms as the starting material. The reaction can be schematically represented:

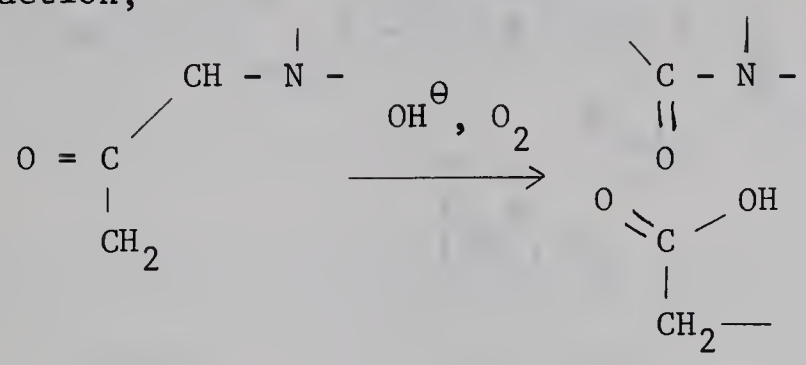


This result implies loss of basicity; the basic nitrogen forms part of a new lactam grouping. It is fully consistent with the enamine character of anhydrolycocernuine.

Earlier in this discussion it has been mentioned that dehydrolycocernuine exchanges three of its hydrogens for deuterium atoms when treated with a mixture of deuterochloric and deuteroacetic acid. In an early attempt to determine the number of enolizable hydrogens present in dehydrolycocernuine, basic conditions ($NaOCH_3$ in methanol-O-d) were employed. No basic product could be isolated from this reaction but ulterior search for the fate of the organic material led to the isolation of an acidic compound. When this matter was investigated in more detail it was found that this carboxylic acid was the main product of the reaction of dehydrolycocernuine with a base. When air was bubbled through the reaction mixture the yield was increased. It seems that both a basic medium and oxygen are essential

for this reaction to take place. The carboxylic acid was identical to the one obtained by $MnO_4^{\ominus}$, $IO_4^{\ominus}$ oxidation of anhydrolycocernuine (the identity was established by comparison of methyl esters).

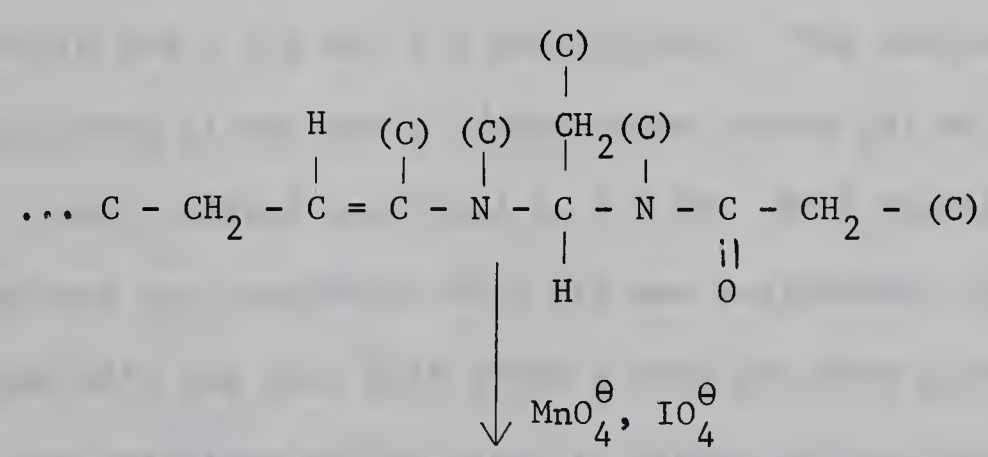
The reaction,



can be rationalized on the basis of the proposed α-aminoketone system* (for a possible mechanism, see below) and in this way confirms our assignment.

The fact that the same carboxylic acid was obtained from the oxidative cleavage of the enamine and from the aerial oxidation of the ketone indicates that dehydration took place without rearrangement and that the assignment of a β-amino alcohol grouping in the alkaloid molecule is correct.

Using the previous scheme [C] these reactions can be schematized as follows: <u>Scheme [D]</u>



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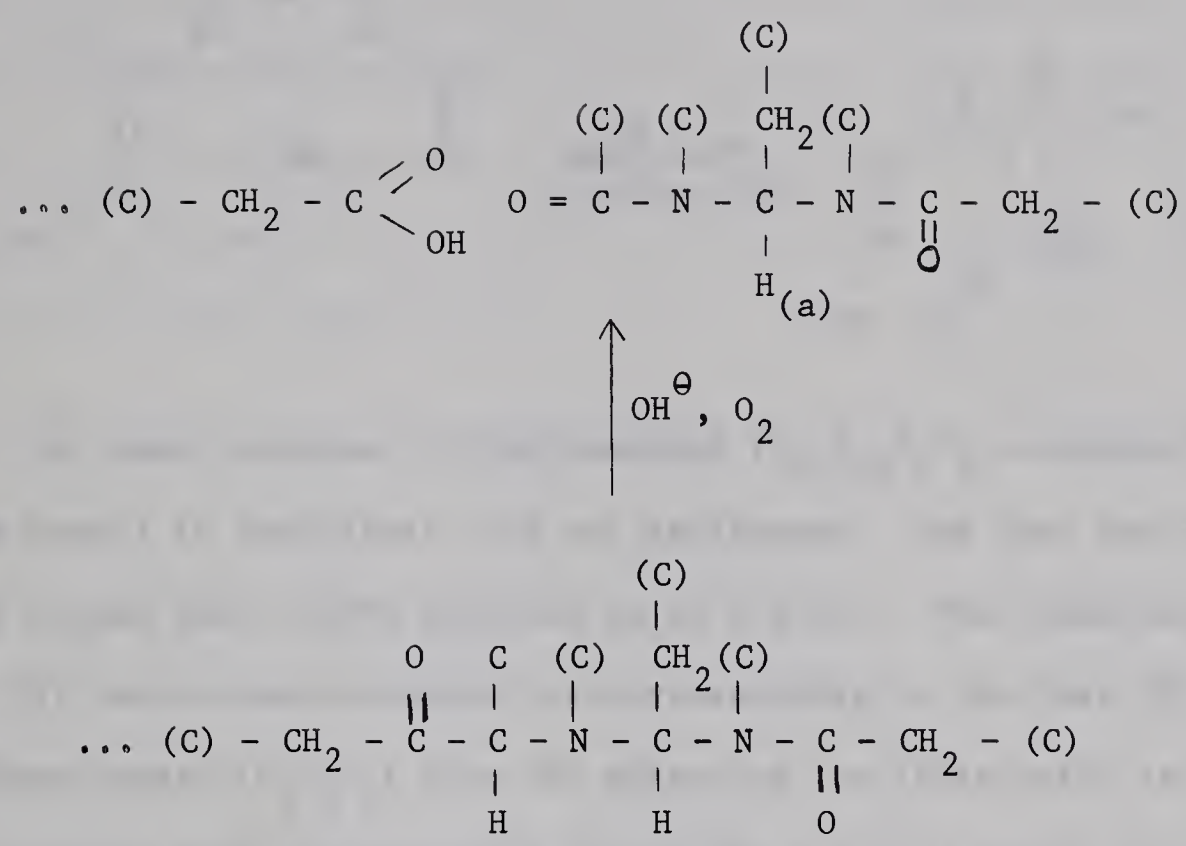
* Other α-aminoketones have been found to be sensitive to air[56,57], their behaviour being analogous to that of α-ketols[58].

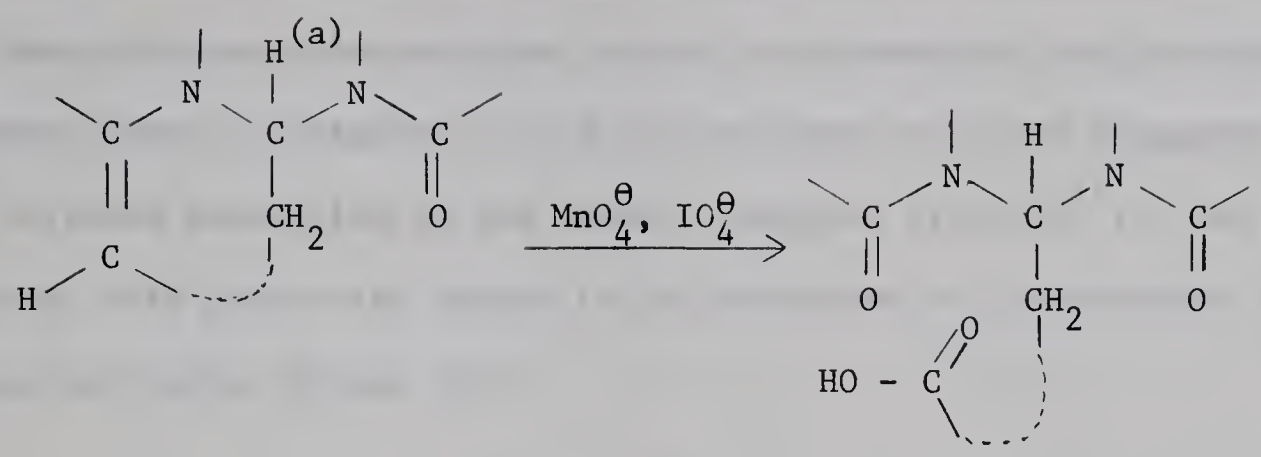
Scheme [D] continued



The point of attachment of the carboxylic acid chain created by the reactions schematically depicted in [D] is apparent from the spectroscopic properties of the methyl ester of the acid.

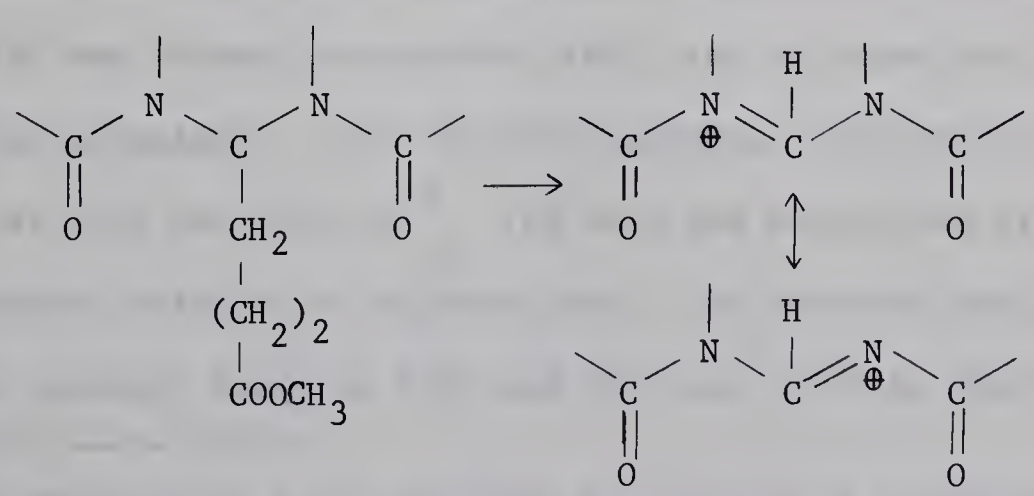
Proton (a) appears as a poorly resolved quartet in dehydrolycocernuine and as a quartet (splittings 11 and 2 cps) in anhydrolycocernuine. The chemical shifts are τ 3.9 and 4.4 respectively. The nuclear magnetic resonance spectrum of the methyl ester shows proton (a) as a clean triplet (splitting 7 cps) shifted downfield to τ 2.68. Both chemical shift and coupling pattern are consistent with its new enviromnent of two lactamic nitrogens and with the fact that since a ring has been cleaved the two protons of the methylene carbon alpha to proton (a) are now magnetically equivalent, (Scheme [E])

Scheme [E]



The mass spectrum of this compound ($C_{17}H_{26}O_4N_2$, molecular wt., 322; mass spec.) is consistent with our assignment. The base peak, and the only strong peak in the spectrum is at m/e 235. The formation of this ion (B) can be best explained as corresponding to the loss of the carbomethoxyl chain ($C_4H_7O_2$) from the molecular ion (this point is confirmed by accurate mass determination of the ion m/e 235; calcd. for $C_{13}H_{19}N_2O_2$: 235.1443; found: 235.1447). The fact that the process leading to the loss of this fragment is so favored relative to other fragmentations suggests that a carbomethoxyl chain ($C_4H_7O_2$) is attached to the carbon atom that is bearing the two nitrogen atoms.

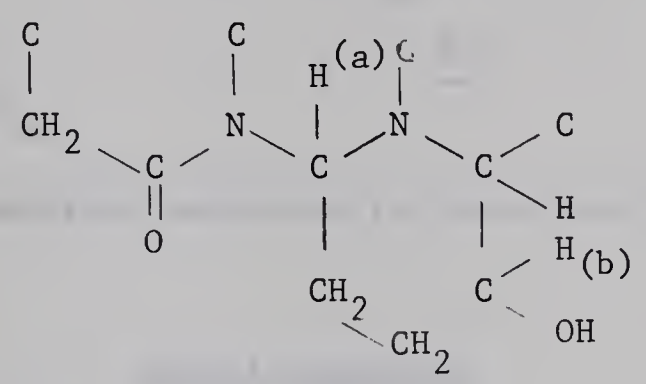
In terms of our partial formulation [E] the process could be represented as follows:



B, m/e 235

This result implies that both by oxidation of anhydrolycocernuine and dehydrolycocernuine we have cleaved a six-membered ring to which the hydroxyl group is attached. This is consistent with the frequency of the infrared absorption of the ketonic compound (1710 cm^{-1}). Our knowledge to this point with regard to the structure of lycocernuine is summarized below (Scheme [F]).

Scheme [F]



To investigate whether or not the aerial oxidation noted above is a general reaction of α-amino ketones we have studied the behaviour of a model compound, 1-ketoquinolizidine, under similar conditions.

1-Ketoquinolizidine (Q) was synthetized following procedures described in the literature[59]. When this compound* was submitted to the same oxidizing conditions as employed with dehydrolycocernuine a carboxylic acid (R) was formed in moderate yield, the nitrogen now forming part of a lactam grouping**. The infrared spectrum (chloroform solution) showed bands at 1710 and 1620 cm^{-1}. The acid was esterified by treatment with an ethereal solution of diazomethane. The infrared spectrum of the ester showed carbonyl bands at 1730 and 1625 cm^{-1} with no absorption in the

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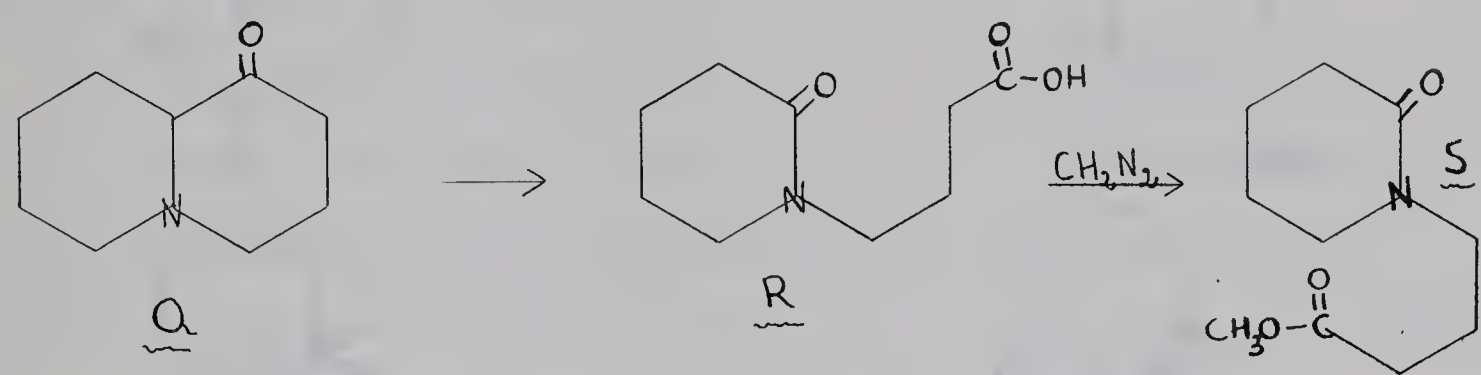
* This material has recently been reported as being sensitive to air[57].

** The lactam formed is perhaps partially hydrolyzed in base. This could account for the low yield.

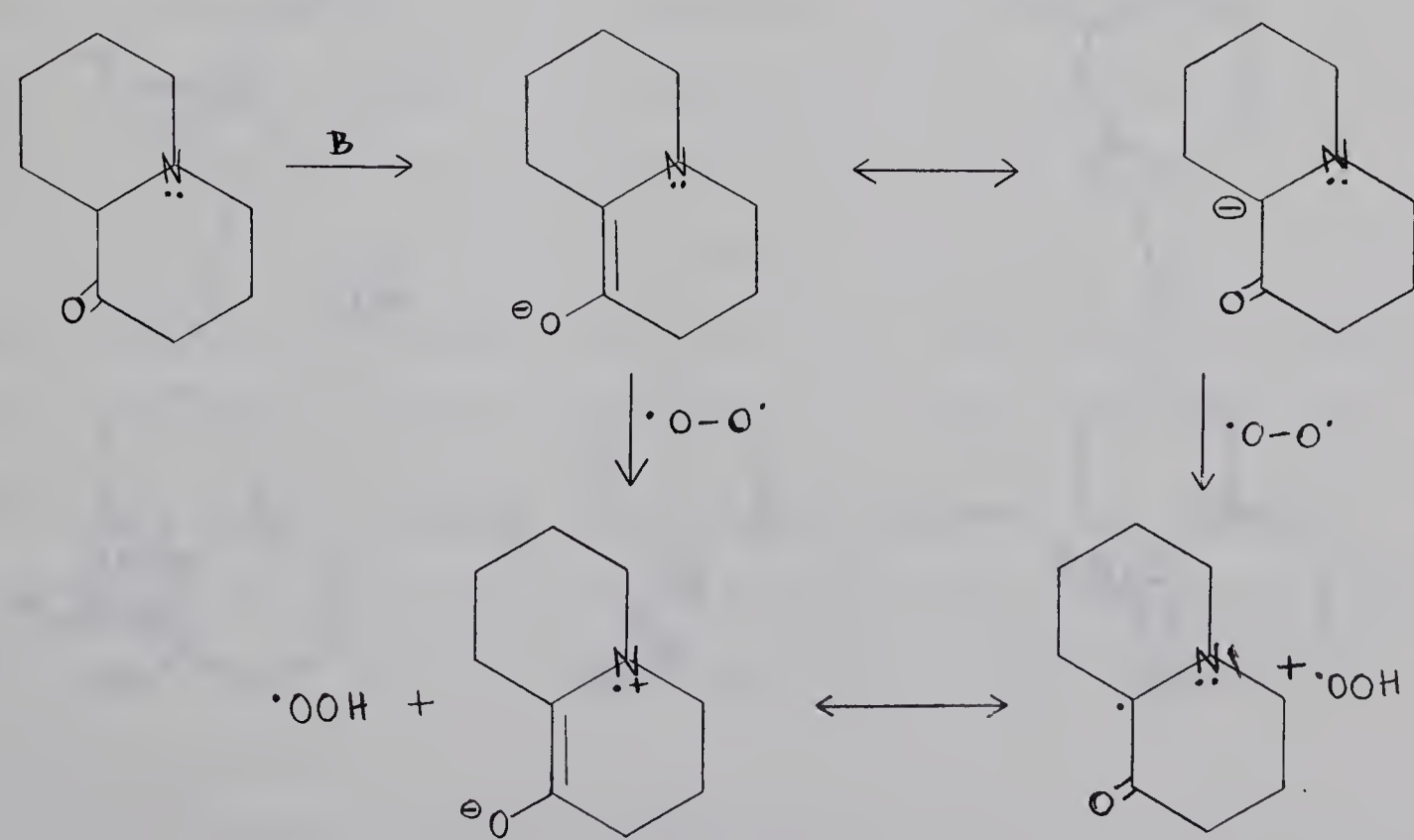
hydroxyl region.

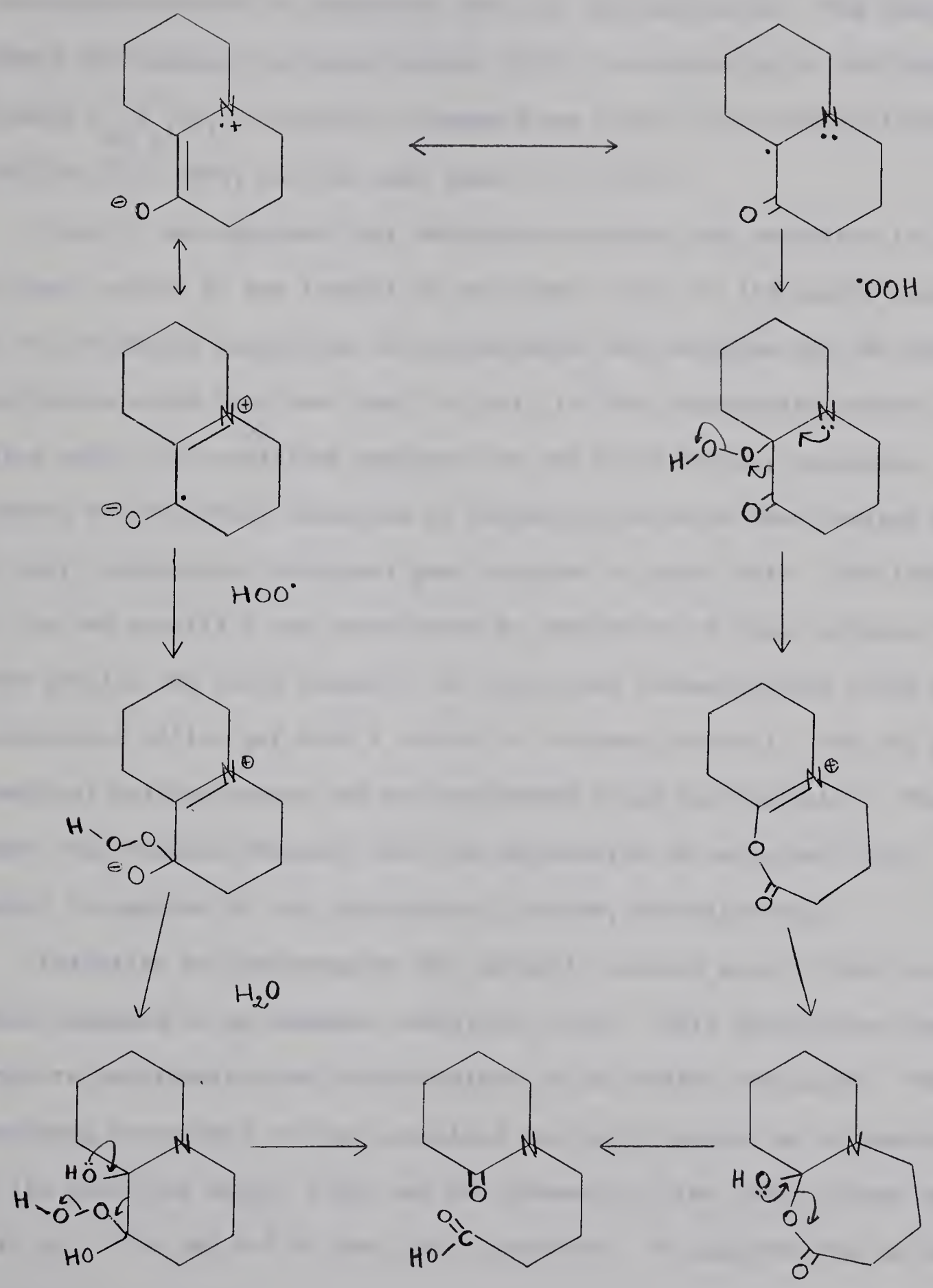
These reactions closely parallel those taking place with dehydrolycocernuine and a similar reaction scheme could be postulated:



A possible, detailed mechanism for this type of aerial oxidation can be written:

Aerial Oxidation




·OOH
HOO·
H_2O

The mass spectrum of the carbomethoxyl derivative obtained from 1-ketoquinolizidine is consistent with our interpretation. The compound showed the expected molecular weight (199), corresponding to the molecular formula $C_{10}H_{17}NO_3$. Important fragments are found at m/e 198 (18), 167 (43), 139 (29), 125 (89), and the base peak at 111 (100).

Once it was apparent that dehydrolycocernuine was sensitive to air in basic media, it was thought in retrospect that the low yields obtained in our attempted conversion of lycocernuine into cernuine via the keto derivative could have been due, in part, to this degradative process taking place under the conditions employed for the Wolff-Kishner reduction. Indeed, Wolff-Kishner reduction of dehydrolycocernuine when carried out in an inert atmosphere (nitrogen) gave cernuine in good yield. The identity of the two materials was established by comparison of their infrared and mass spectra and their behaviour on thin-layer chromatography (both on alumina and silica gel with a variety of solvent systems). The two had identical melting points and an undepressed mixed melting point. This meant that results obtained from the degradation of cernuine* could safely be applied to the lycocernuine problem, and vice versa.

Oxidation of lycocernuine with mercuric acetate gave a crude product which appeared to be somewhat sensitive to air. This product was acetylated prior to purification and characterized as the acetyl derivative. The resulting acetylated product contained two acetyl groups as evidenced by its molecular weight (360) and the presence of two three proton singlets at τ 7.76 and 8.0 in the n.m.r. spectrum. It appeared that we had

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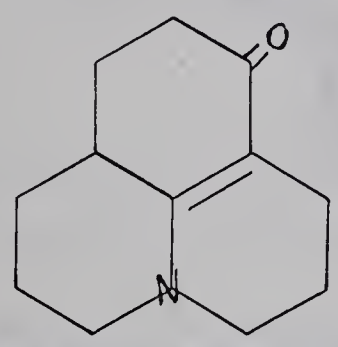
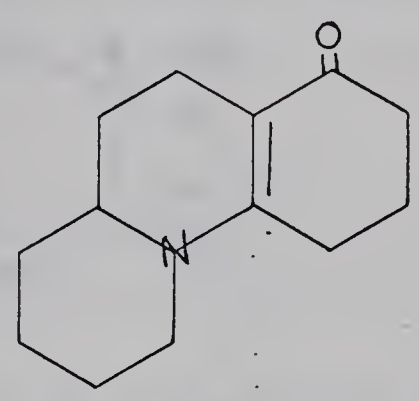
* J. K. Jenkins. Private communication.

acetylated the hydroxyl group and also an enamine function. This latter possibility was suggested by the fact that the acetylated compound displayed absorption in the ultraviolet at 322 mμ (log 3.43). Compounds such as the ones below absorb in the u.v. at $\lambda_{max.}$ 315 mμ,[60] and at $\lambda_{max.}$ 316 mμ[61] respectively





These absorptions are associated with the system

$$-\underset{|}{\ddot{N}}-C=C-\overset{O}{\overset{\|}{C}}-$$

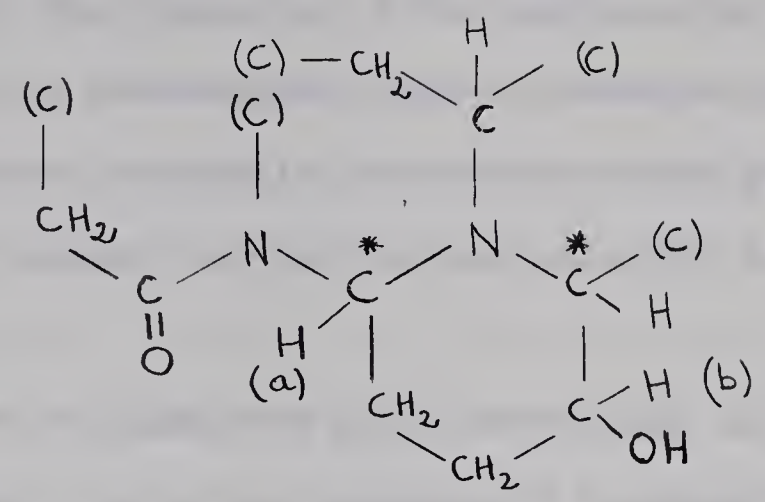
The nuclear magnetic resonance spectrum of the diacetylated derivative showed two one-proton low field signals at τ 4.3 and 5.1. Taking scheme [F] as the basis for our reasoning, those two signals have been assigned to protons (a) and (b) in [F]. The absence of any other signals in the region of olefinic or enamine absorption implies that the double bond must be tetrasubstituted and hence that the situation illustrated below must hold.

$$-N-\underset{\underset{H}{|}}{\overset{|}{C}}-CH_2-C- \longrightarrow -N-\overset{C}{\overset{|}{C}}=\overset{C}{\overset{|}{C}}-\underset{\underset{O}{\|}}{C}-CH_3$$

Scheme [G] shows the partial structure enlarged to account for the

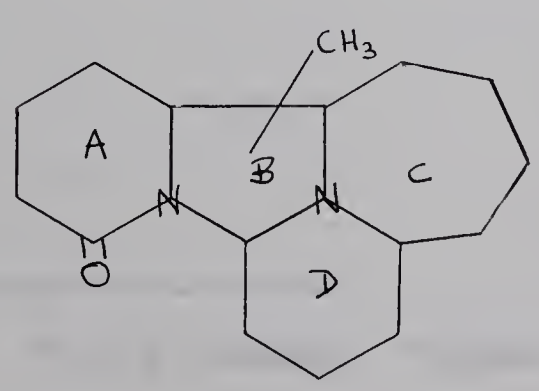
structural features just depicted.

Scheme [G]

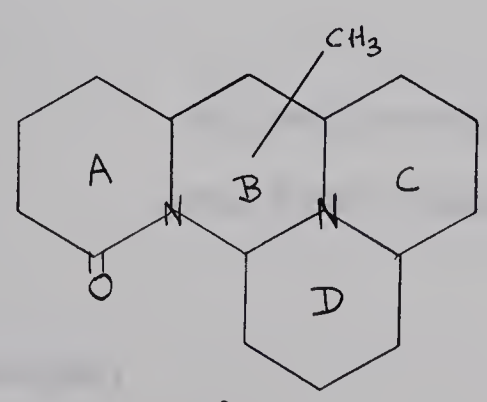


As it has been mentioned above, protons (a) and (b) appear at τ 4.3 and 5.1 in the n.m.r. spectrum of the diacetylated product; that is they have almost the same chemical shifts as in O-acetyllycocernuine. This rules out the possibility that oxidation had occurred toward either of the starred carbons in [G].

In partial structure [G] we have tentatively located ten of the sixteen carbon atoms, plus the nitrogens and the oxygenated functions. Since the compound has to be tetracyclic, the hydroxyl group is located on a six-membered ring and the lactam ring is probably six-membered, structures such as 1 and 2 accomodate the deductions made to this point.



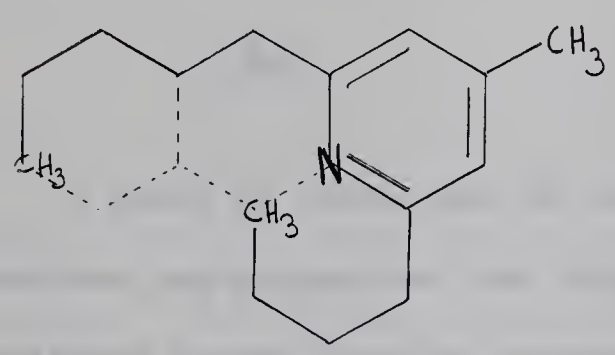
1.



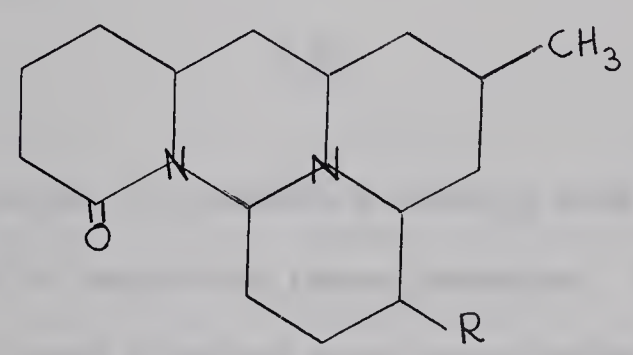
2.

Since there was no information about the size of rings B and C, it was impossible at this stage to make a distinction between these structures. As we will see later in the discussion of the mass spectral data, the structure containing four six-membered rings is consistent with such data, while some of the results, especially those obtained with products in which ring B has been cleaved, are hard to reconcile with a structure such as 1.

Dehydrogenation of cernuine with palladium-charcoal at 300° carried out in our laboratories*, led to the isolation of 2-n-butyl-4-methyl-6-n-pentylpyridine (3) in 10% yield[49]. If we assume that the C-4 methyl group in the 2-n-butyl-4-methyl-6-n-pentylpyridine represents the methyl group in the cernuine-lycocernuine skeleton, then structure 2 offers a ready explanation of the dehydrogenation results as shown:



3.



2a, R=H, cernuine

2b, R=OH, lycocernuine

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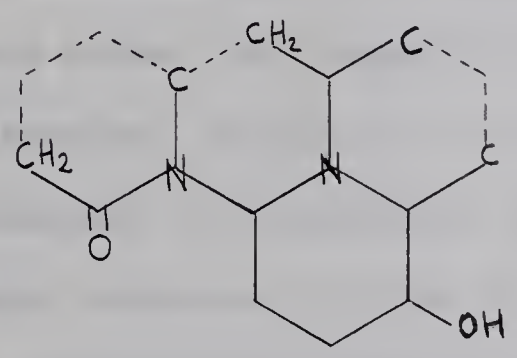
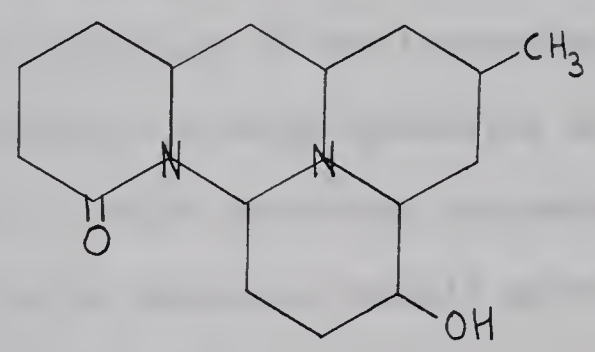
* J. K. Jenkins. Private communication.

This pyridine accounts for all except one of the carbon atoms in the original alkaloid. The missing carbon in the pyridine (3) corresponds to the lactam carbonyl group. It was found that dehydrogenation of dihydrodeoxycernuine*, in which the lactam carbonyl is reduced to methylene, gave 2-n-butyl-4-methyl-6-n-hexylpyridine which accounts for all the carbon atoms present in the natural compound.

Both the dehydrogenation results and partial structure [G] are consistent with structure 2b for lycocernuine.



[G]



2b

It would be convenient at this point to summarize briefly some of the reactions and properties that helped to establish these formulae. The functional groups (lactam, hydroxyl) were inferred from spectroscopic evidence, and further confirmed by chemical means (acetylation, hydride reduction, oxidation, etc.). Once the tertiary nature of both nitrogens was established n.m.r. evidence was used to determine the relative

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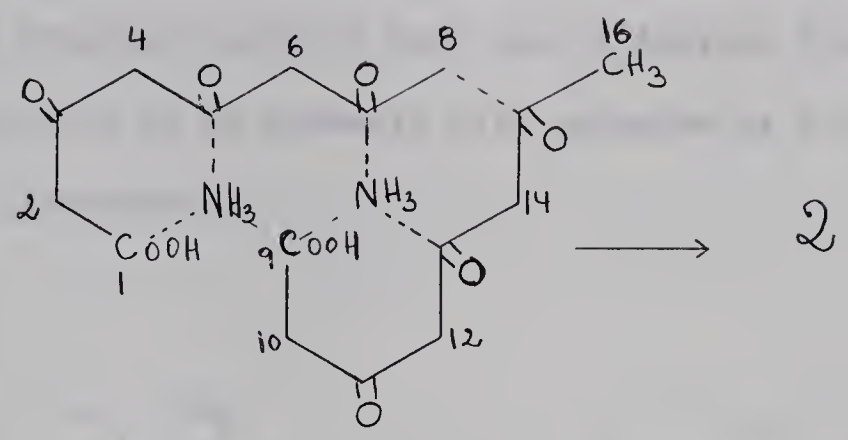
* J. K. Jenkins. Private communication.

position of the nitrogens to one another. The hydroxyl group was linked to the basic nitrogen by consideration of the properties of the dehydration and oxidation products of lycocernuine. This evidence favored the presence of a beta amino alcohol system in the lycocernuine molecule. Results obtained from the mercuric acetate oxidation of lycocernuine and from deuterium exchange experiments carried out with lycocernuine and with the keto derivative were used to further extend our knowledge of the carbon skeleton of the alkaloid. Lycocernuine was successfully related to cernuine. The pyridines isolated from the dehydrogenation of cernuine and dihydro-deoxycernuine are consistent with the proposed partial structure and, as discussed below, the skeleton can be derived from 2 triketooctanoic acid chains, the suggested biogenetic precursors of the Lycopodium alkaloids. Structure 2 is thus a reasonable working hypothesis for the structure of lycocernuine and cernuine. These structures represent a major departure from the type of molecular skeletons usually encountered in alkaloids from plants of the Lycopodium genus.

The biogenetic scheme suggested by Conroy[28] for the Lycopodium alkaloids is still applicable although no connection with lycopodine or a lycopodine-type of alkaloid (such as those described in the introduction) is immediately obvious. The proposed skeleton for lycocernuine-cernuine can be easily derived[49] from two chains of polyketooctanoic acid, as depicted below, by condensation with two molecules of ammonia and proper adjustment of the oxidation levels.

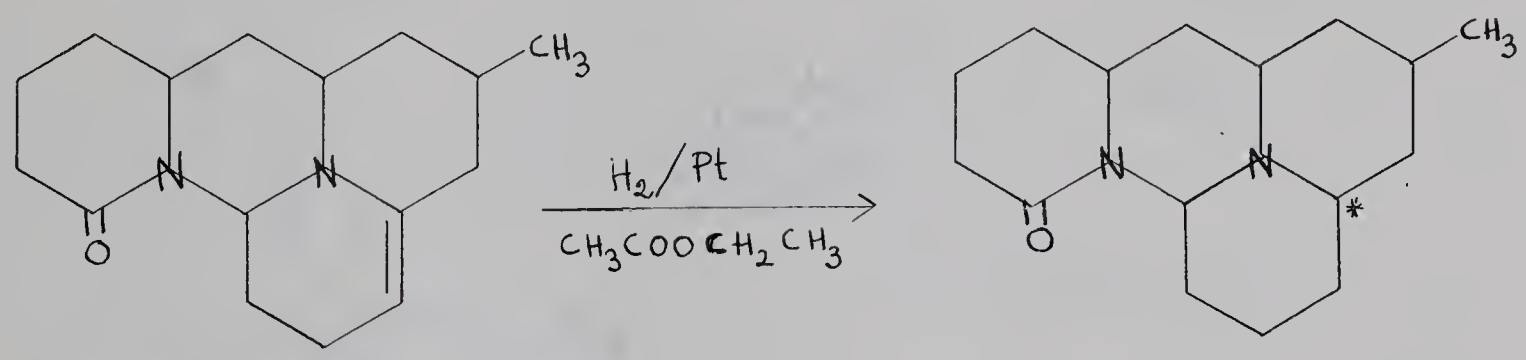


The fact that one could readily arrive at structure 2 from precursors suggested to be involved in the biogenesis of other Lycopodium alkaloids was considered a strong point in favor of this structure, as mentioned above.

In these particular alkaloids the two hypothetical units that form the final structure are linked by a single C-C bond (C-8 to C-15) which suggests the possibility that the first C-C bond formed in the biogenesis of all the Lycopodium alkaloids may be the C-8 to C-15 bond, since the formation of this bond is implicated in the proposed biogenetic scheme discussed in the Introduction.

Further degradative work carried out with lycocernuine can be readily rationalized on the basis of the proposed structure (2) and serves to confirm the validity of the assignment. When anhydrolycocernuine (4) was hydrogenated in the presence of Adam's catalyst in ethyl acetate a small amount of cernuine, together with a new product (5), was obtained.

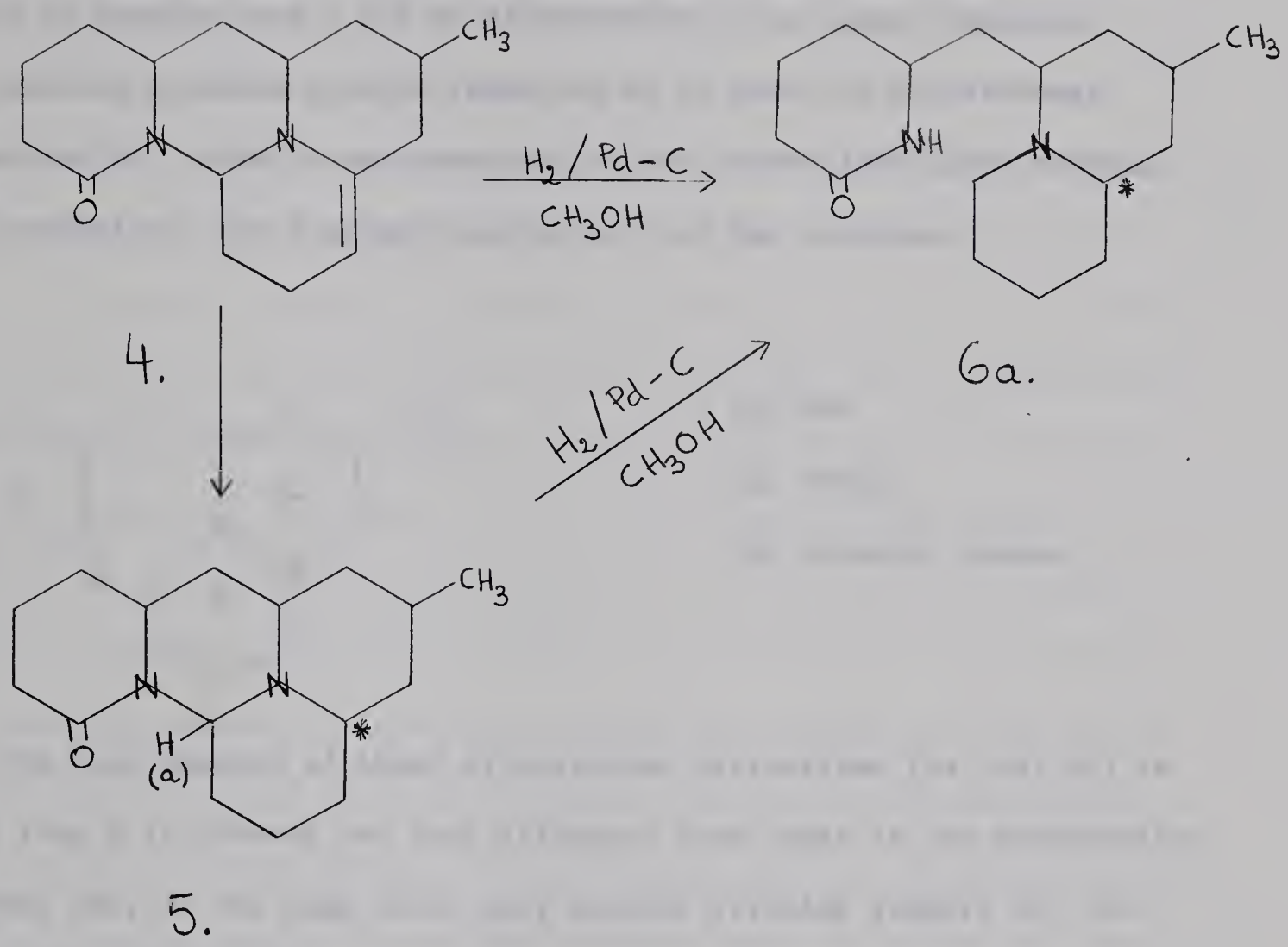
This new compound had a mass spectrum almost identical with that of cernuine but it showed different chromatographic behaviour. Its infrared and nuclear magnetic resonance spectra were also different from those of cernuine. We consider (5) to be epimeric with cernuine at C-13 and have named this compound allocernuine.



4. 5.

(*) Epimeric center

Hydrogenation of a methanolic solution of anhydrolycocernuine (4) with palladium-on-charcoal as the catalyst results in the uptake of two moles of hydrogen with the formation of dihydroallocernuine. Under these same conditions, allocernuine takes up one molecular equivalent of hydrogen, with the formation of the same dihydroallocernuine (6a). These reactions are formulated as follows:

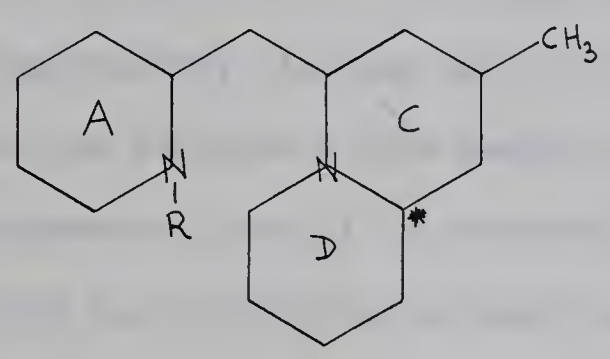


(*) Epimeric center

That the second mole of hydrogen causes cleavage of the C-9 to lactam nitrogen bond is evident from the following facts: The infrared spectrum of the product shows a band at 3400 cm^{-1} (NH) in the infrared; the product fails to acetylate when treated with pyridine and acetic anhydride. Both these facts are consistent with our assumption and at the same time make highly improbable that some bond other than the C-9 to lactam nitrogen (e. g., the C-9 to basic nitrogen or C-13 to basic nitrogen) has been cleaved. That C-9 is involved in the cleavage is also

evident from the fact that in the n.m.r. spectrum of the hydrogenation product (dihydroallocernuine, 6a), the low field proton (a) present at τ 4.54 in cernuine and τ 5.0 in allocernuine is no longer observed.

Lithium aluminum hydride reduction of 6a gave the dihydrodeoxy derivative 6b. When 6b was submitted to the Eschweiler-Clarke methylation conditions, the N-methyl derivative (6c) was obtained.

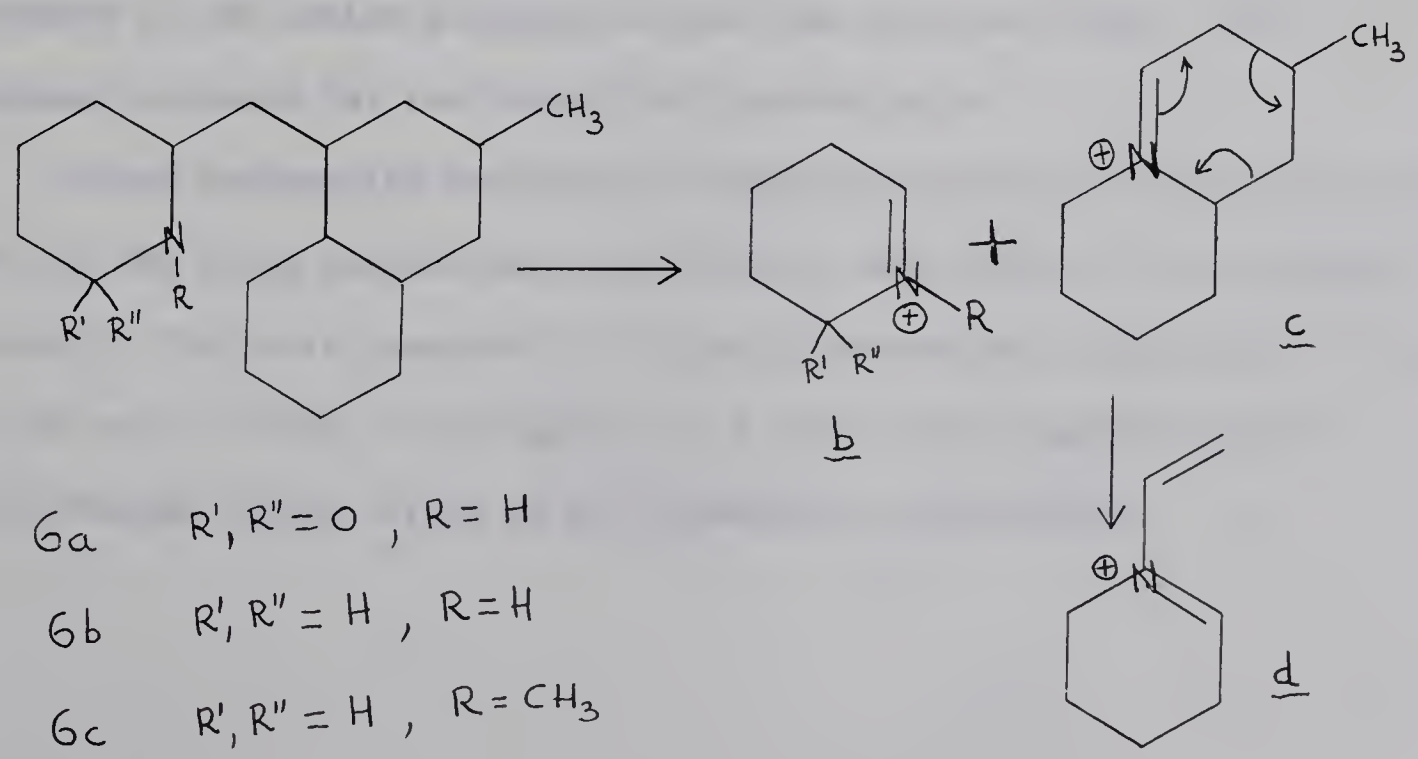


6b, R=H

6c, R=CH_3

(*) Epimeric center

The mass spectra of these allocernuine derivatives (6a, 6b, 6c) in which ring B is cleaved are very different from those of the tetracyclic compound and, at the same time, they provide striking support for the structural assignments.



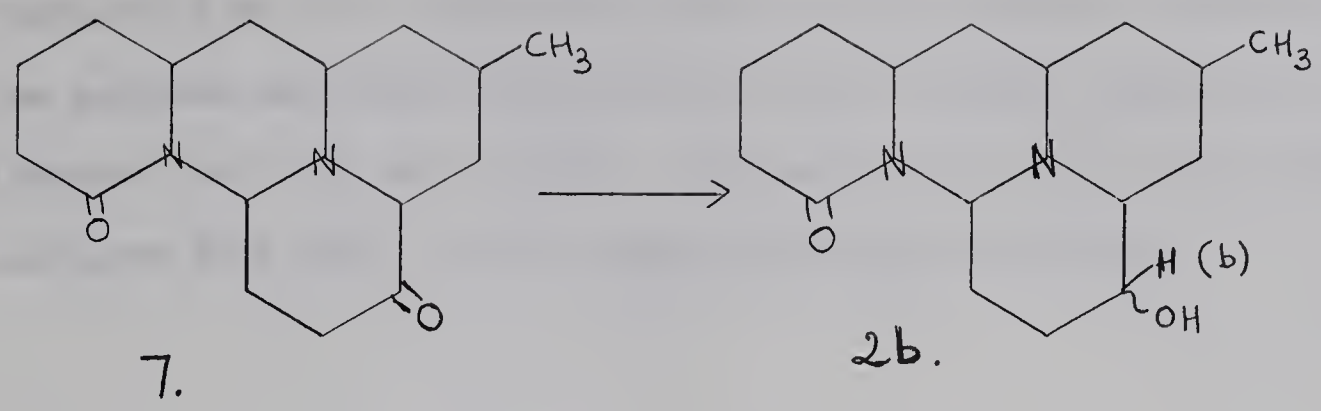
6a R', R'' = O , R = H

6b R', R'' = H , R = H

6c R', R'' = H , R = CH_3

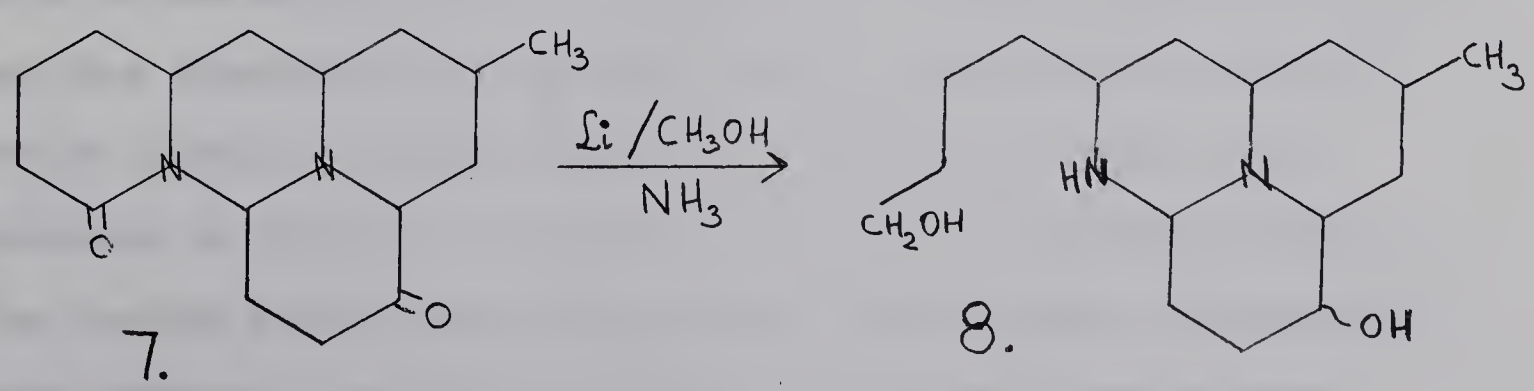
Each one of these compounds (6a, 6b, and 6c) exhibits the expected molecular ion in their mass spectra. The base peak at m/e 152 (c) is common to the three of them, in addition each shows a very intense peak at m/e 110 (d) corresponding to the loss of propene from the ion m/e 152 via a retro-Diels-Alder type of reaction[62]. This fragmentation is supported by the appearance of a metastable peak at m/e 80.1, calculated for 152 → 110 79.6, and provides evidence in support of the positioning of the C-methyl group relative to the methylene group (C-6) linking the two heterocyclic ring systems. It does not, of course distinguish between C-14 and C-15 for the location of the methyl group. The presence of these two important fragments (c and d) in the mass spectra of 6a, 6b, and 6c practically rules out possible structures such as 1 for lycocernuine and indicates that ring C is indeed six-membered. In addition compound 6b shows an intense peak at m/e 84 (b R=R'=R"-H), and compound 6c an intense peak at m/e 98 (b, R'=R"=H, R=CH_3). The peak at m/e 98 for dihydroallocernuine (6a) is not particularly strong but this presumably reflects the decreased tendency of the lactam nitrogen to bear the positive charge. This fragment accounts for the 2-piperidyl portion of 6.

Sodium borohydride reduction of ketolycocernuine (7) gives lycocernuine (2b) as the major product but also yields a small amount of the epimeric alcohol. The n.m.r. spectrum of O-acetyllycocernuine shows proton (b) at τ 5.06 with a width at half-height of 5 cps, which suggests that in lycocernuine proton (b) is in the equatorial conformation.

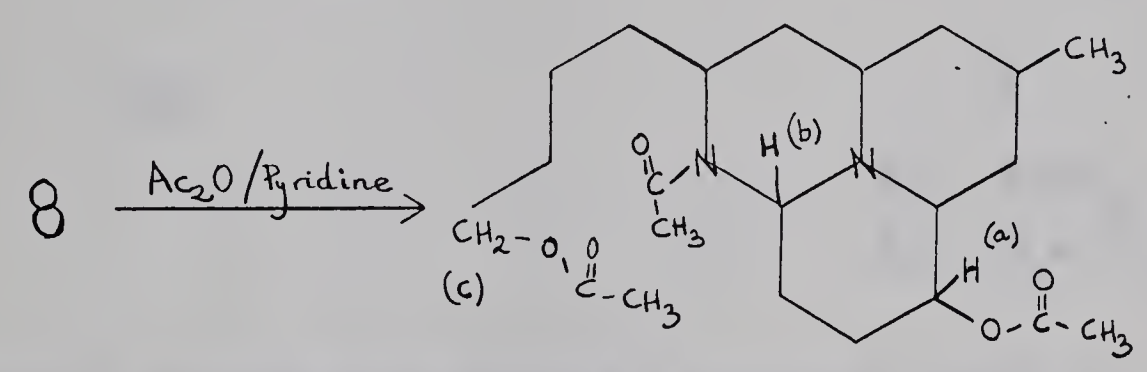


It was considered that it would be of interest to compare the spectroscopic and chemical properties of lycocernuine (or suitable derivatives such as the acetate) with those of the epimeric alcohol. Sodium borohydride reduction of dehydrolycocernuine (7) gave only a very small amount of the desired epimeric alcohol. Reduction of the ketone with dissolving metal-alcohol was expected to afford the equatorial alcohol as the major product. Dehydrolycocernuine was, therefore, subjected to reduction with lithium-methanol in liquid ammonia. The crude product of this reduction (two components, t.l.c.) showed an increase of six units in molecular weight with respect to the starting ketone. The infrared spectrum displayed OH and NH absorption and it was devoid of absorption in the carbonyl region.

It thus appeared that reductive cleavage of the lactam ring had accompanied reduction of the ketone (7 → 8)

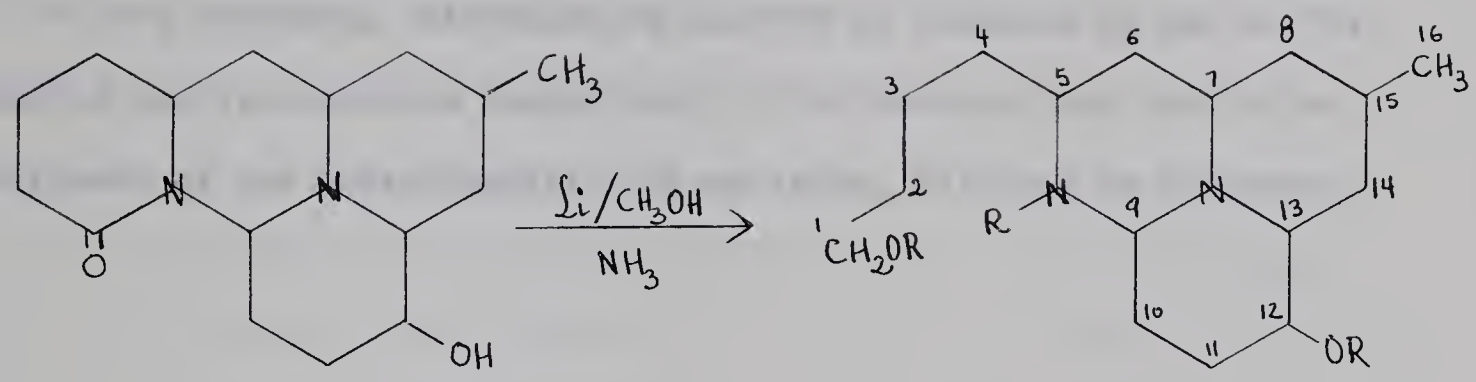


The main product of this reduction (~ 90% of the mixture) was separated from the accompanying epimer as the triacetyl derivative (9a). The purified derivative showed bands in the infrared spectrum at 1720 (acetate) and 1620 cm^{-1} (amide). The mass spectrum and n.m.r. spectrum indicated that three acetyl groups had been incorporated.



The n.m.r. spectrum of 9a showed a three proton singlet at τ 7.9 and a six proton signal at τ 7.95 due to the acetyl groups, and a methyl doublet at τ 9.1. Proton (a) appears as a sextet (J = 4.5 cps) at τ 4.8, proton (b) is present as a poorly resolved multiplet at τ 5.26 and protons (c) appear as a triplet (2H) at τ 5.9.

The same procedure was applied to lycocernuine itself. Reduction with lithium-alcohol in liquid ammonia gave a single product 9b that did not show absorption in the carbonyl region, but showed OH (3600 cm^{-1}) and NH (3200 cm^{-1}, broad) absorption. The molecular weight (282) indicates an increase of four mass units over the starting material. The cleavage product was acetylated with pyridine-acetic anhydride at room temperature and again a triacetyl derivative 9c was obtained.



2b

9

9b, R = H

9c, R = C(=O)-CH3

The n.m.r. spectrum of 9c displays a six-proton singlet at τ 7.9 and a three-proton singlet at τ 6.9. The methyl doublet is at τ 9.1. A one-proton signal at τ 5.1, appearing as a poorly resolved multiplet with a width at half-height of 6 cps, can be assigned to the C-12 proton (a). The C-9 proton (b) appears as a multiplet at τ 5.3 and the C-1 methylene (c) as a triplet at τ 5.9. This data is consistent with our formulation for 9c. It also suggests that 9a and 9b are epimeric compounds at C-12. The same product (9b) was obtained, as a minor component, in the reduction of dehydrolycocernuine.

At this point we felt that considering the difficulty encountered in obtaining the alkaloids in reasonable quantities, further proof of the structural assignment could be best obtained by means of total synthesis*. We therefore turned our attention to the problem of defining the stereochemistry of the six asymmetric centers present in lycocernuine.

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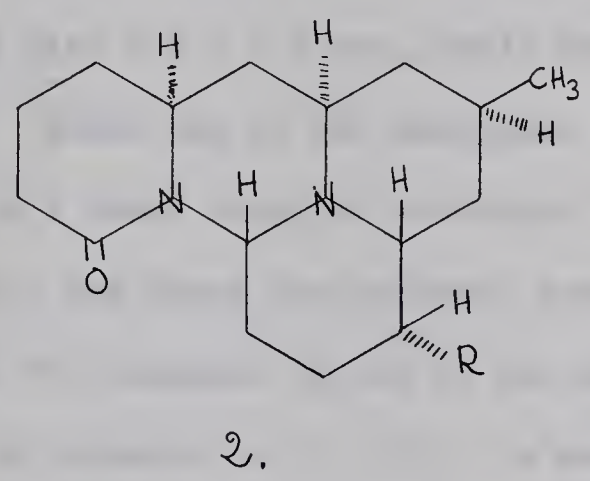
* This has now been achieved (K. Piers. Ph.D. Thesis. Univeristy of Alberta, 1966)

Stereochemistry

In the preceding discussion we arrived at formulae 2a and 2b for cernuine and lycocernuine respectively. The results that led to the assignment of the stereochemistry shown below, will now be discussed



2.

2a, R=H

2b, R=OH

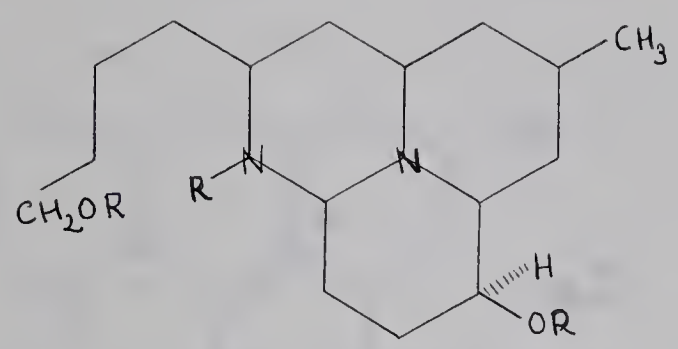
2c, R=$OCOCH_3$

The starting point for the determination of the relative configuration of the alkaloids was the assignment of the configuration of the hydroxyl group in lycocernuine.

The proton geminal to the acetoxyl group in O-acetyllycocernuine gives rise to signal at τ 5.06 in the n.m.r. spectrum of 2c (see Fig. 2). Although this signal appears as a poorly resolved multiplet, measurement of the half-height width of the signal ($w_{1/2}$ = 5 cps) indicates that the proton responsible for this signal is an equatorial proton since it has been shown[35] that the half-height width for axial protons in unresolved

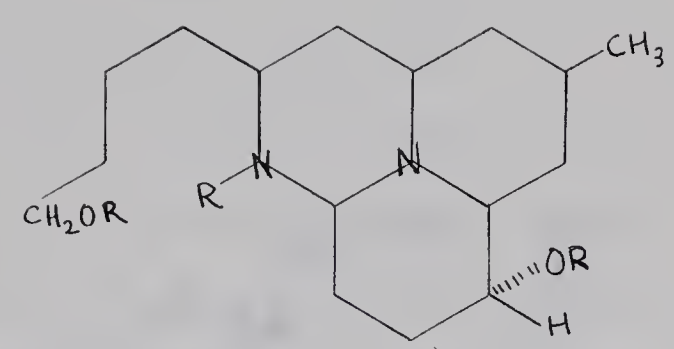
multiplets falls in the range 15 - 30 cps whereas the range for equatorial protons is 5 - 12 cps. It was possible to confirm this assignment by spin-decoupling experiments. Simultaneous irradiation at τ 6.8 (presumably the C - 13 proton) causes the signal at τ 5.06 to collapse to a triplet (splitting 2.5 cps) while irradiation at τ 8.13 (presumably the C - 11 methylene group) causes it to collapse to a doublet, splitting 2.0 cps. The vicinal coupling constants between axial and equatorial protons usually fall in the range 2.5 - 3.5 cps, between two equatorial protons the range is also 2.5 - 3.5 cps, while between two axial protons it is 10 - 11 cps[63]. Since one of the hydrogens at C-11 must necessarily be axial, the lack of a large coupling involving the C-12 hydrogen indicates that it is equatorial, and hence the hydroxyl group is axial.

The preparation of compounds 9a and 9c has been described earlier. These two products are epimeric at C - 12. In the n.m.r. spectrum of 9c, prepared by reduction of lycocernuine with Li - CH_3OH in ammonia (axial conformation of the hydroxyl group at C - 12), the C - 12 proton is a multiplet (width at half-height 6 cps) at τ 5.1, while in the n.m.r. spectrum of 9a, the major product of the reduction of dehydrolycocernuine with Li - CH_3OH in ammonia (equatorial conformation of the hydroxyl group at C - 12), the C - 12 proton is a sextet, half-height width ($w_{1/2}$ 25 cps), at τ 4.8. These results confirm the assignment of an axial conformation to the hydroxyl group of lycocernuine



9a

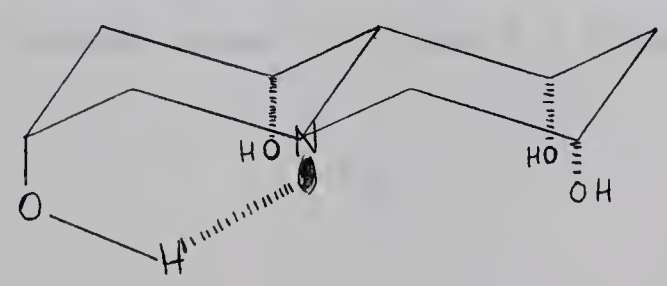
R= C(=O)-CH3



9c

R= C(=O)-CH3

Lycocernuine shows free OH absorption (3620 cm^{-1}) in the infrared.

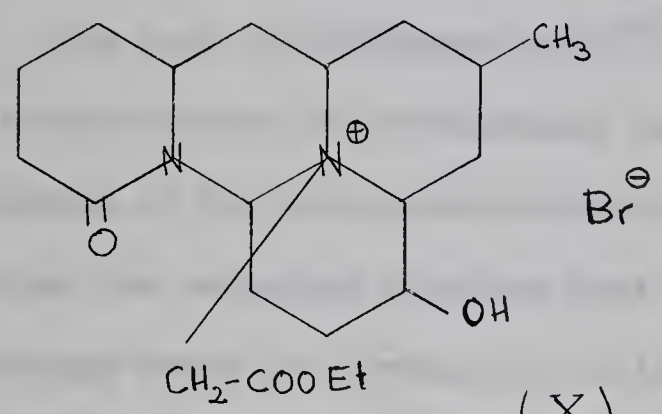


(Y)

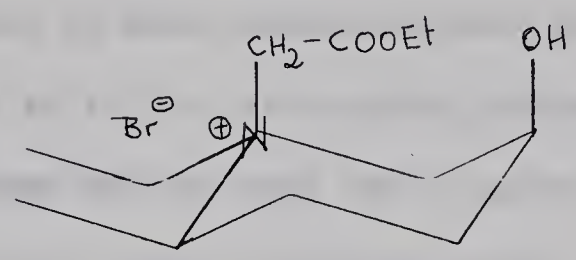
In a trans-quinolizidine system, an axial hydroxyl group with a 1-3 relationship to the nitrogen is necessarily in a position which favors intramolecular OH to N hydrogen bonding (Y)[79].

The lack of hydrogen-bonding to nitrogen[80] suggests that rings C and D in lycocernuine form a cis-quinolizidine system in which the axial hydroxyl group is located in the ring to which the lone pair of the nitrogen bears an equatorial relationship.

Consistent with this assignment was the fact that the quaternary salt (X) formed between lycocernuine and ethyl bromoacetate failed to lactonize when hydrolyzed with 4N hydrobromic acid



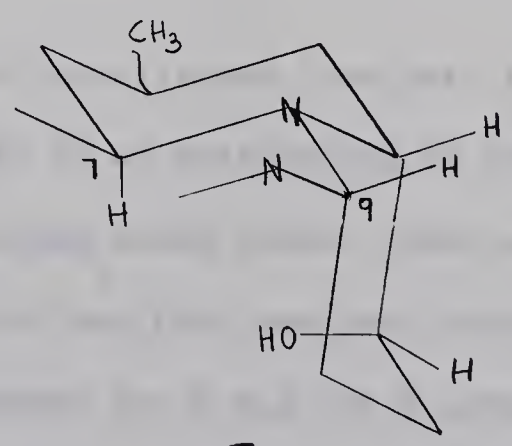
(X)



(A)

Compounds of type A readily form lactones under these conditions[64].

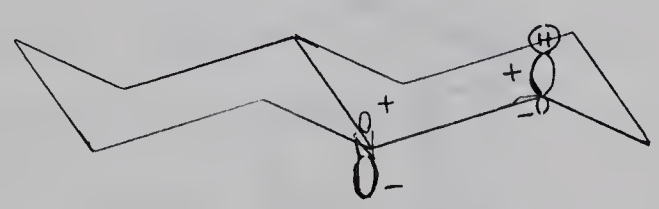
These results are summarized in partial structure I in which the stereochemistry of carbon atoms C - 7 and C - 9 has not been defined as yet.



I

The fact that C - 9 has the configuration depicted in I is apparent from the n.m.r. spectrum of lycocernuine (as well as cernuine) and various other derivatives in which the C - 9 proton appears as a quartet with a large splitting of 10 - 12 cps and a small splitting, 2 - 4 cps. The large coupling constant must be due to an axial-axial coupling[63].

The lack of "Bohlmann bands"[65] in the 2700 - 2800 cm^{-1} region of the infrared spectra of lycocernuine and cernuine is also consistent with the presence of the cis-quinolizidine system as in I. If, in a quinolizidine system the nonbonded electron pair on nitrogen and at least two α-carbon-hydrogen bonds are arranged in a trans-coplanar manner, Bohlmann bands (a complex absorption in the 2700 - 2800 cm^{-1} region) appear in the infrared spectrum. A trans-quinolizidine fusion of rings C and D in lycocernuine should give rise to absorption in this region. According to Hamlow and Okuda[66] in systems such as the quinolizidine (Z)

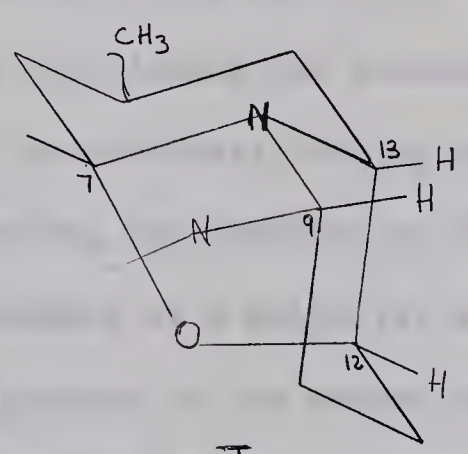


(Z)

in which the direction of the nitrogen lone pair is fixed, partial participation of the lone pair in an antibonding σ* orbital between the α-carbon and the axial hydrogen takes place. This participation allows some overlap between the σ* and the lone pair orbitals generating some double bond character between the N and the α-carbons and simultaneously increasing the electron density at the α-carbon axial proton, thereby lowering the frequency of the stretching vibration of those C-H bonds.

Such an interaction can only be expected in the axial-hydrogen direction since a σ* of the α-carbon-equatorial hydrogen orbital lies in a plane perpendicular to the lone pair.

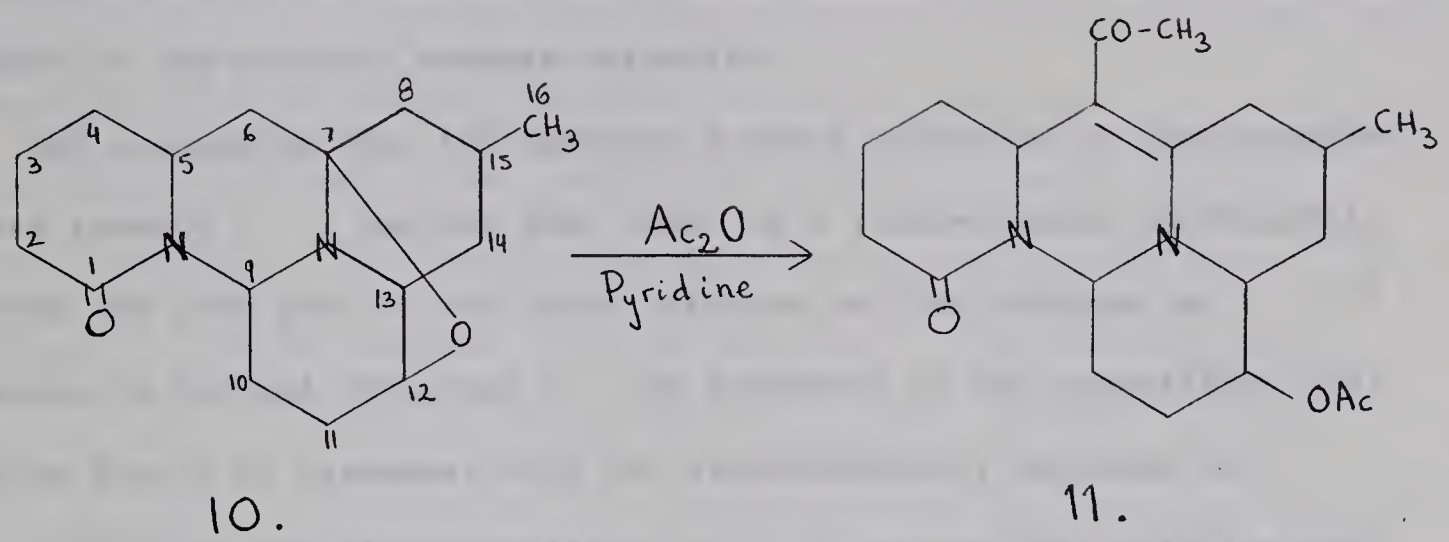
The assignment of configuration at C - 7 was based on the following observations. When lycocernuine was oxidized with mercuric acetate in aqueous acetic acid, the dehydro product obtained ($C_{16}H_{24}O_2N_2$, mass spectrometry) did not exhibit OH or ketonic absorption in its infrared spectrum. Its n.m.r. spectrum was devoid of olefinic or enamine absorption. Since a new unsaturation is created and no OH absorption is observed, an oxazolidine structure (part structure II) was assigned to this oxidation product.



II

The fact that this compound still retained the C - 9 proton with its characteristic quartet structure at τ 4.20 eliminates the possibility that the oxidation could have taken place towards C - 9. Two other directions C - 13 and C - 7 are still left for consideration. The fact that the initial mercuric acetate oxidation of lycocernuine took place toward C-7 is suggested by the spectroscopic data obtained with the acetyl

derivative of the oxazolidine 10.

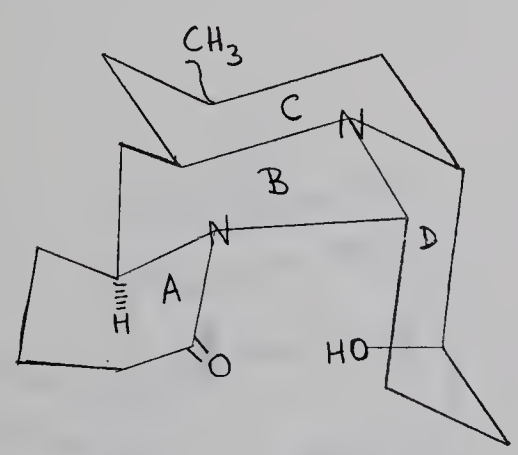


As has been mentioned earlier (Discussion and Results), both mass spectrometry (molecular weight 360) and n.m.r. spectrometry (three proton singlets at τ 7.76 and 8.0)indicated the presence of two acetyl groups. One of the acetyl groups is obviously forming part of an O-acetyl function, as the lack of OH absorption, the presence of a band at 1720 cm^{-1} in the infrared, and the presence of a multiplet at τ 5.1 in the n.m.r. spectrum testifies. The placing of the second acetyl group at C - 6 rather than C - 8 is due mainly to the fact that decoupling experiments carried out with 11 showed that the C-16 methyl group appears to be coupled to a proton at τ 8.05. This chemical shift is in the usual range for the chemical shift of the C - 15 methine proton (e. g., τ 8.1 in O-acetyl-lycocernuine), and is not compatible with an allylic proton. For the same reason the enamine double bond cannot be located between C - 13 and C - 14. Since there is no indication of the formation of a ketonic compound formation of a C - 12 to C - 13 olefin (actually an enol)

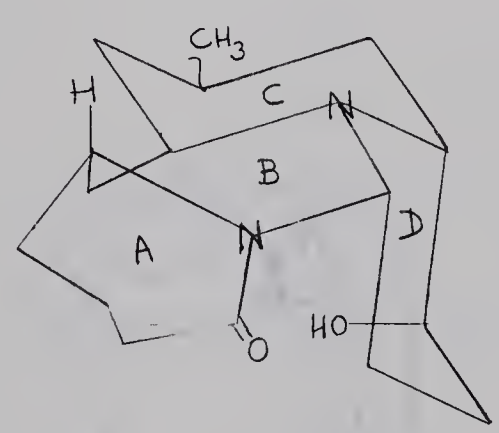
is excluded. Therefore it is reasonable to eliminate C - 13 as a possible direction for the oxidation and consider that the oxazolidine (10) arises by cyclization of the immonium salt formed (toward C - 7) as the initial product of the mercuric acetate oxidation.

The conclusion that the mercuric acetate oxidation of lycocernuine* occurs towards C - 7 implies that there is a trans-diaxial relationship between the lone pair on the basic nitrogen and the hydrogen at C - 7[76], as shown in partial structure I. The formation of the oxazolidine (10) is also nicely in agreement with the stereochemistry depicted in I.

Turning to the stereochemistry at C - 5, we are left with the two alternatives shown below.



2c



2d

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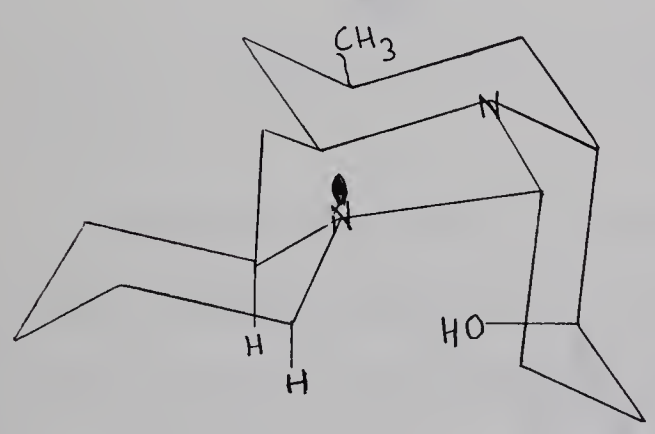
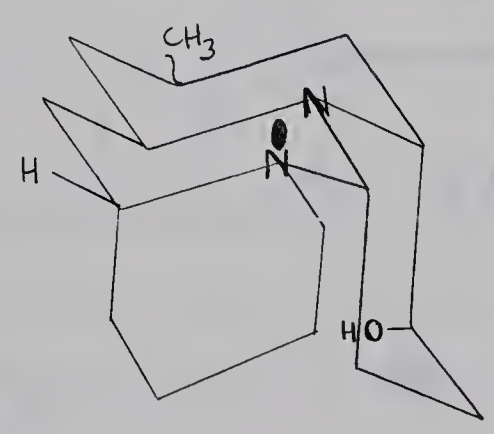
* Similar oxidation of cernuine also takes place towards C - 7. J. K. Jenkins. Private communication.

In one of these two possibilities (2d) ring B is forced into a twist conformation if the planarity of the amide group is to be preserved and this seems to be the case since all the lactams in these series show normal infrared carbonyl stretching vibrations at 1620 - 1640 cm^{-1}.

Neither lycocernuine nor cernuine show Bohlmann bands in their infrared spectrum. The dihydrodeoxyderivatives of these alkaloids (obtained by lithium aluminum hydride reduction of the lactam group), however, exhibit the characteristic set of bands in the 2700 - 2800 cm^{-1} region (see Fig. 3) indicative of a trans-quinolizidine like system with at least two α-hydrogens trans-diaxial to the lone electron pair of the newly produced basic nitrogen. This result is compatible with structure (12a), (derived from 2c), but not with 12b (derived from 2d) and defines the configuration at C - 5.



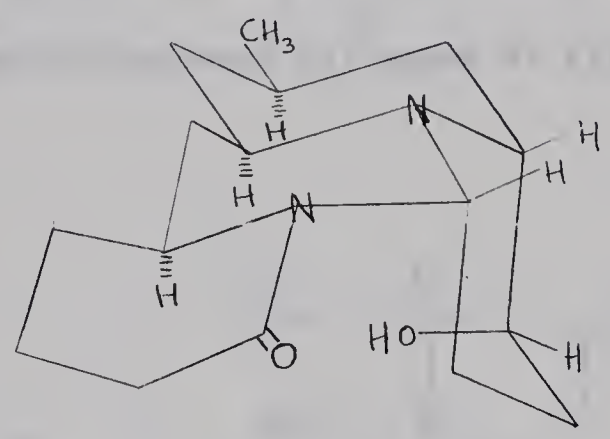
12a



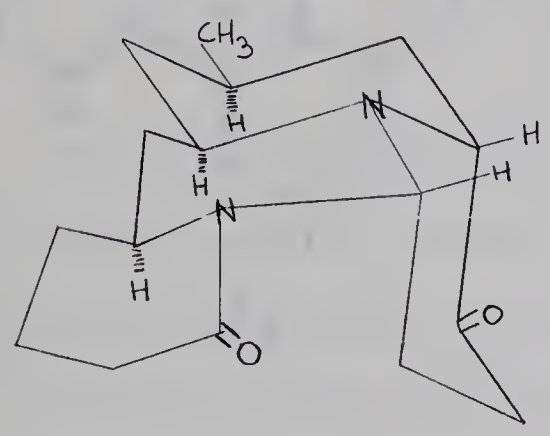
12b

With regard to the configuration of C - 15, it is not possible at this point to make a rigorous assignment. The results obtained in the sodium borohydride reduction of dehydrolycocernuine (7a) in which a

substantial amount (20%) of the epimeric lycocernuine is obtained tend to favour an equatorial configuration for the methyl group, since an axial methyl at C - 15 would considerably hinder the attack of the borohydride from the rear (Y), necessary for the formation of the epimeric alcohol (12).

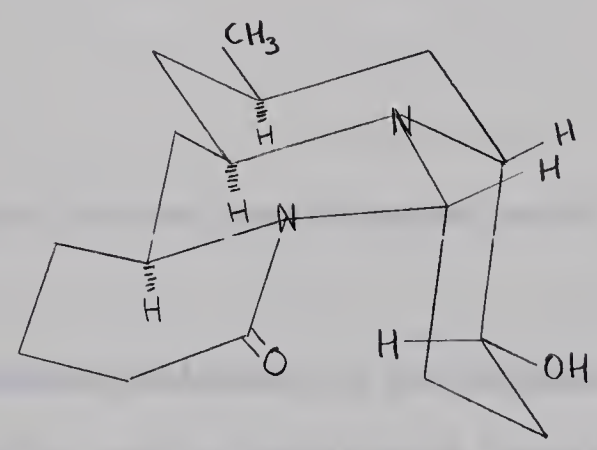


2c

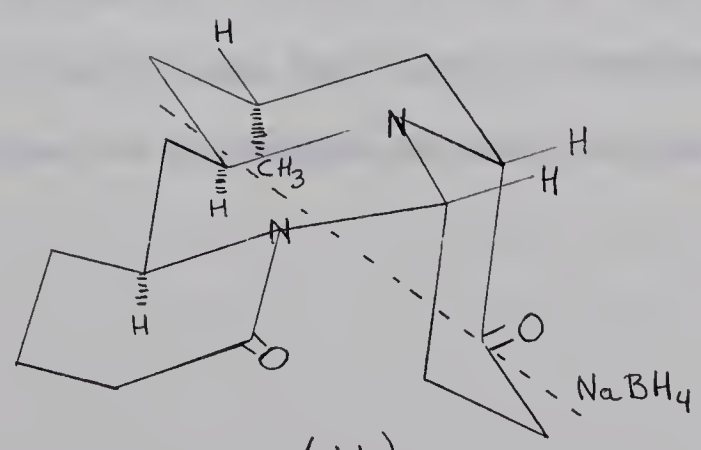


7a

$\xrightarrow[CH_3OH]{NaBH_4}$



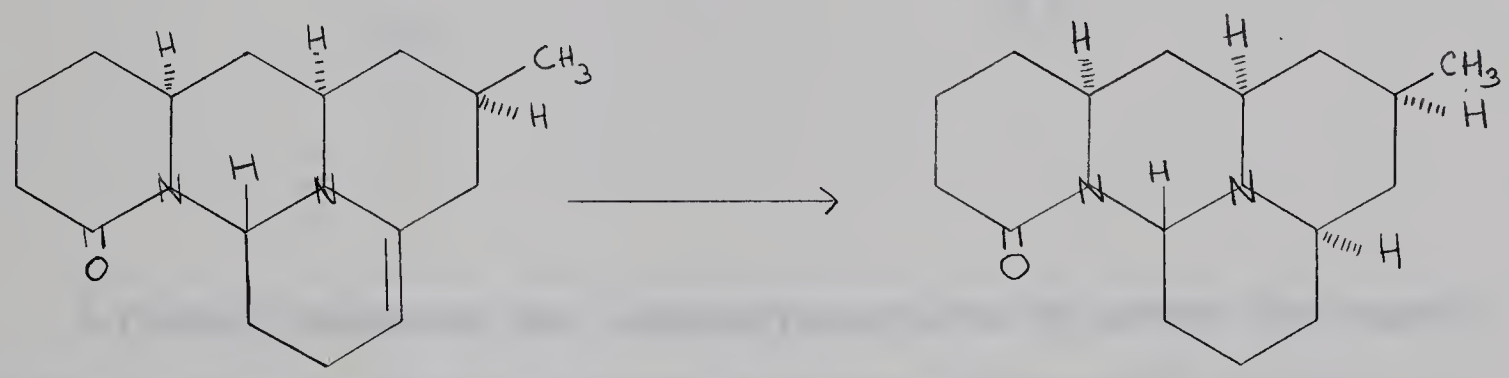
2e



(y)

Lycocernuine is represented by 2c, and the epimeric alcohol by 2e.

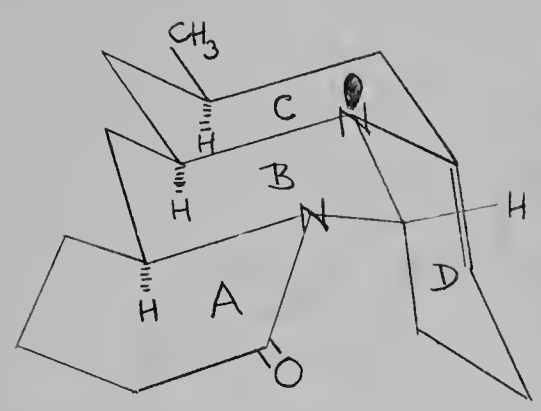
As mentioned previously, catalytic hydrogenation of anhydrolycocernuine (4) in ethyl acetate produces a compound, allocernuine, that is isomeric with cernuine at C - 13. Allocernuine is formulated as 5. In addition to 5 a small amount of cernuine (2a) is obtained. The almost exclusive formation of 5 by hydrogenation of the anhydrocompound (4) seems at first sight surprising,



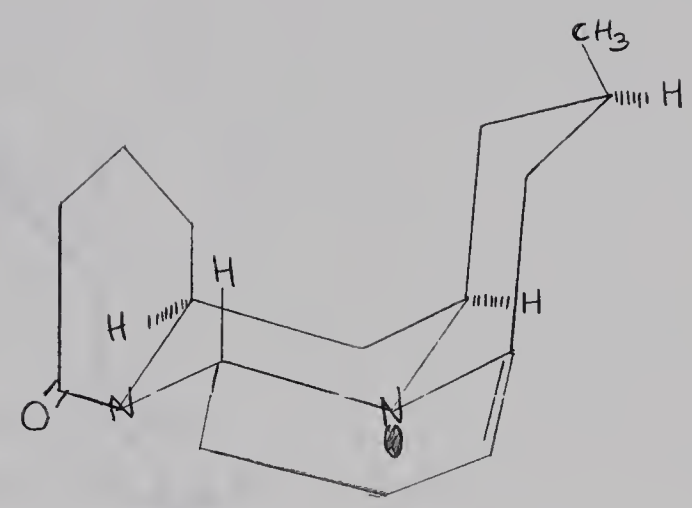
4. 5.

since the top side of the molecule seems to be the less hindered side (see 4a).

However, 4a is not the only conformation available to the molecule. Since the basic nitrogen is conformationally mobile the form 4b resulting from inversion of the nitrogen must also be considered. In 4b approach of the catalyst from the bottomside is more favored.

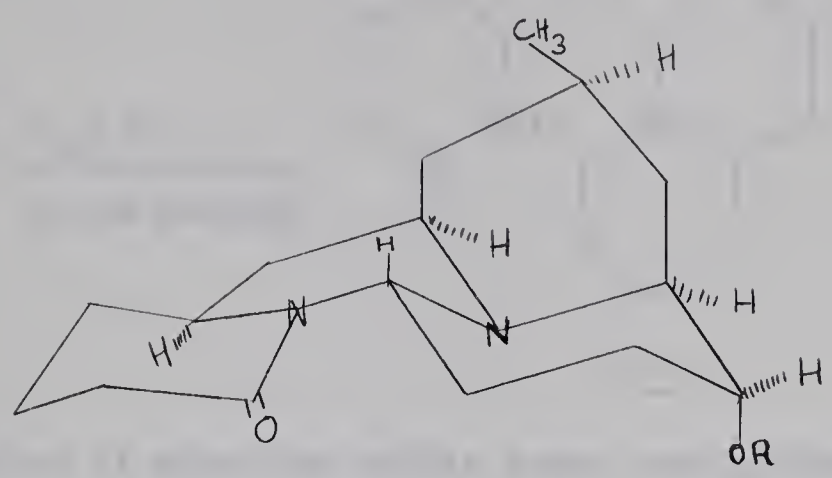


4a



4b

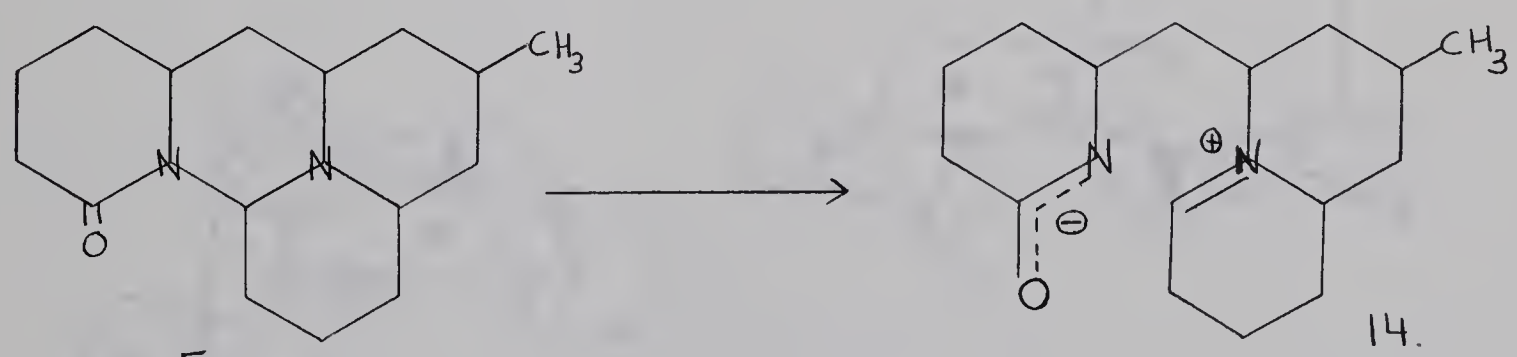
A further indication that anhydrolycocernuine (4) exists (or reacts) in this conformation (4b) was obtained by Brown hydration[67] of (4). When anhydrolycocernuine was reacted with diborane and the adduct was treated with hydrogen peroxide in alkaline solution, the main product of the reaction was a secondary alcohol ($C_{16}H_{26}O_2N_2$, mass spectrometry) which was different from both lycocernuine (2c) and epilycocernuine (2e). Obviously this product must differ from the natural series in the configuration at C - 13 and therefore must possess the same configuration as allocernuine (5) at C - 13. It appears that the hydroxyl group of this new product (13) (lycoallocernuine) is in the axial configuration [in the n.m.r. spectrum of O-acetyllycoallocernuine (13a) the proton geminal to the acetyl group consist of a poorly resolved multiplet ($w_{1/2}$ 6 cps) at τ 5.34]. Addition of diborane to a double bond takes place in all cis-fashion[68]; to obtain an axial alcohol with the allo-configuration at C - 13 addition to the olefin must take place through a configuration such as 4b. Lycoallocernuine is, therefore represented by (13).



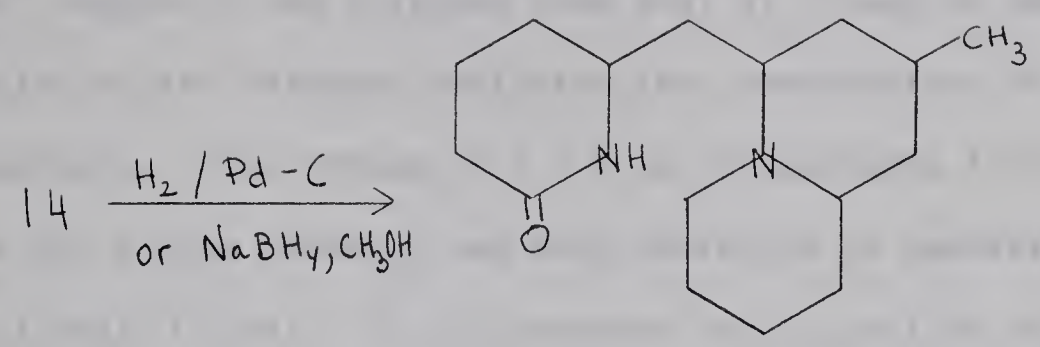
13, R = H

13a, R = CO-CH3

As mentioned previously, allocernuine (5), undergoes facile reductive cleavage of the hexahydropyrimidine system when hydrogenated in methanolic solution in the presence of palladium-charcoal catalyst or when treated with sodium borohydride in methanolic solution. This cleavage presumably takes place via a zwitterionic intermediate such as 14,

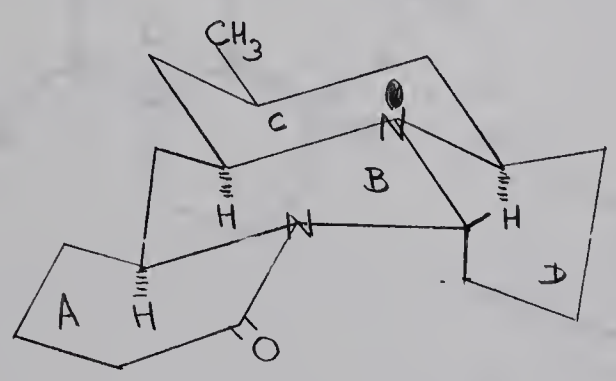


5. 14.

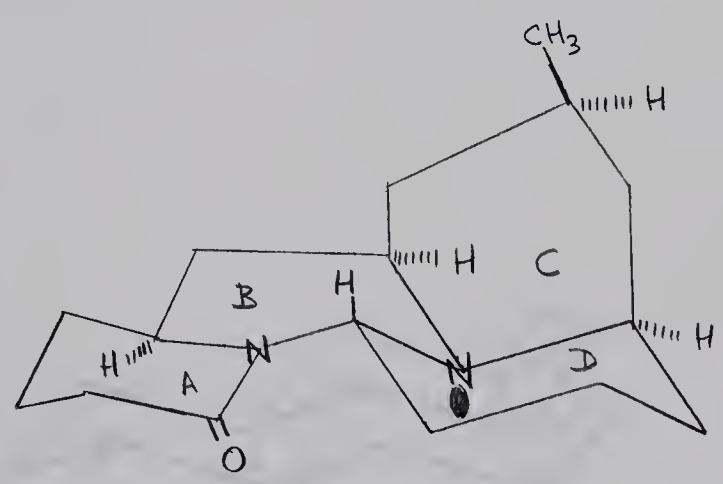


6a

Inspection of molecular models shows that allocernuine is a strained compound in which one of the rings is forced into a twist conformation. Considering the conformational mobility of the basic nitrogen, allocernuine (5) may exist predominantly in the form 5a in which ring D is in the twist conformation or alternatively, in the nitrogen inverted form 5b, in which ring B is in the twist conformation.



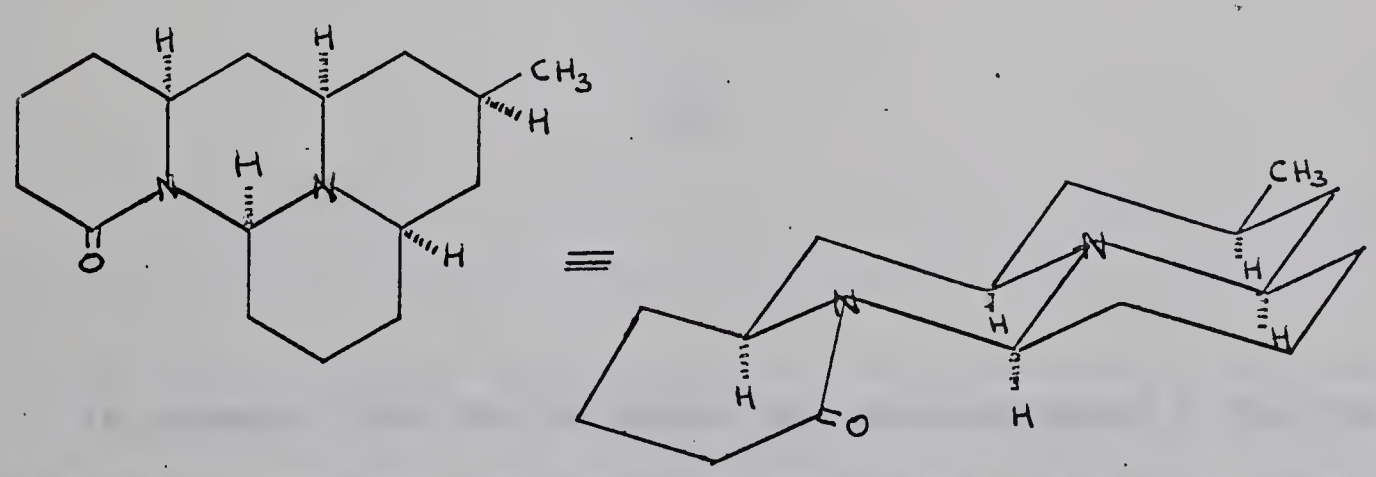
5a



5b

Allocernuine does not show absorption (Bohlmann bands) in the 2700 - 2800 cm^{-1} region of the infrared (see Fig. 4). Lack of absorption in this region of the infrared indicates that conformation 5b is favoured by allocernuine. The proton at C - 9 in lycocernuine (2c) lies in the plane of the lactam carbonyl and must therefore be deshielded by the carbonyl[69] (chemical shift τ 4.54). In allocernuine this signal is shifted upfield to τ 5.01; since in a conformation such as 5b, the C - 9 proton is twisted somewhat out the carbonyl plane this data favors conformation 5b for allocernuine as well. This result also means that both allo- and lyco-allocernuine (13) adopt the same conformation. In the n.m.r. spectrum of O-acetyl-lycoallocernuine (13b) the C - 9 proton is at τ 5.03.

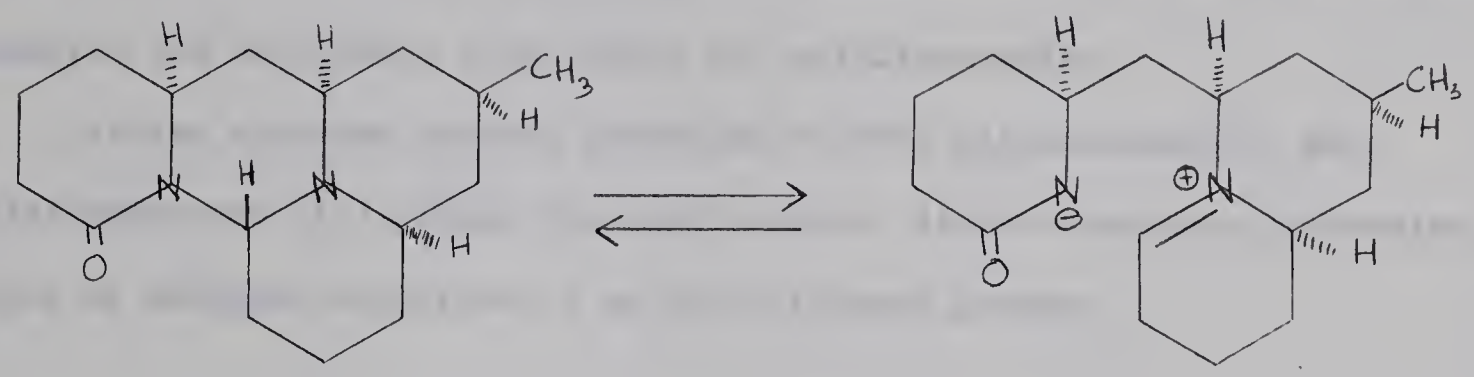
When allocernuine is refluxed in methanol in the absence of reducing agents, isomerization takes place and new substance called epiallocernuine (15) is obtained.



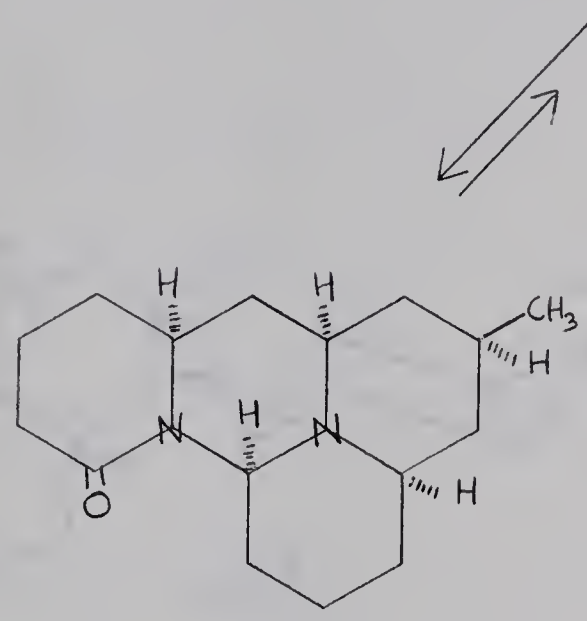
15.

As is apparent from the conformational drawing (15), epiallocernuine should be thermodynamically more stable than allocernuine, and isomeriza-

tion presumably occurs via the zwitterionic intermediate (16), as shown below.



5. 16.

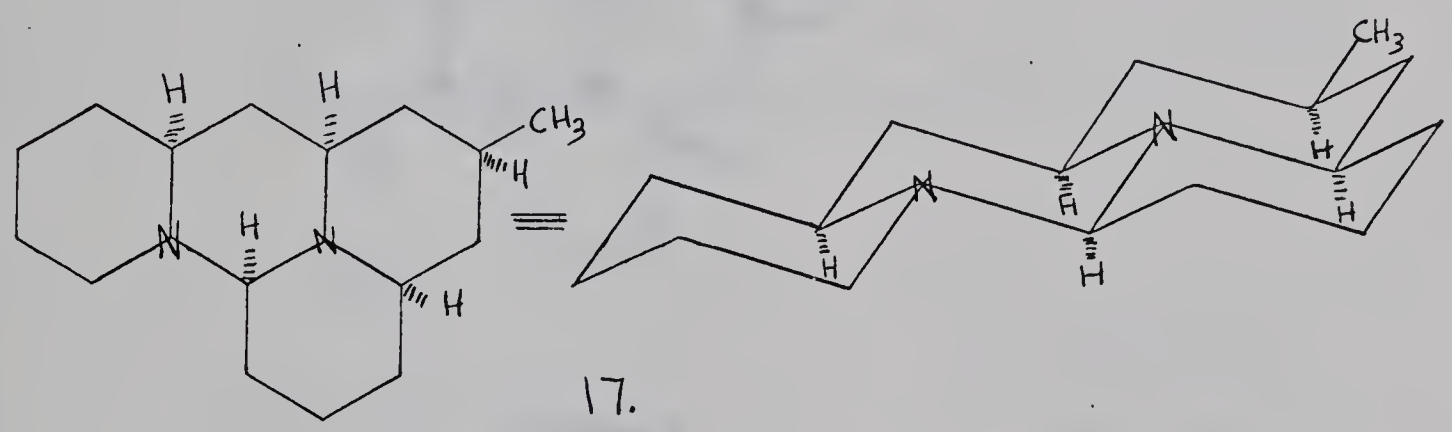


15.

In agreement with the assignment for epiallocernuine is the fact that in its n.m.r. spectrum the absorption due to the C - 9 proton forms part of a multiplet at τ 6.2 - 6.6, the highest chemical shift encountered for this proton in the tetracyclic compound series with an unreduced lactam group. This chemical shift obviously reflects the fact that now the C - 9 proton is perpendicular to the plane of the lactam group[69].

Also, as required by its stereochemical formula (15), epiallocernuine exhibits Bohlmann bands (see Fig. 5). The molecular weight (260) and most important fragments present in the mass spectrum of epiallocernuine follow the pattern encountered in both cernuine and allocernuine; all this data supports the assignment given above for epiallocernuine.

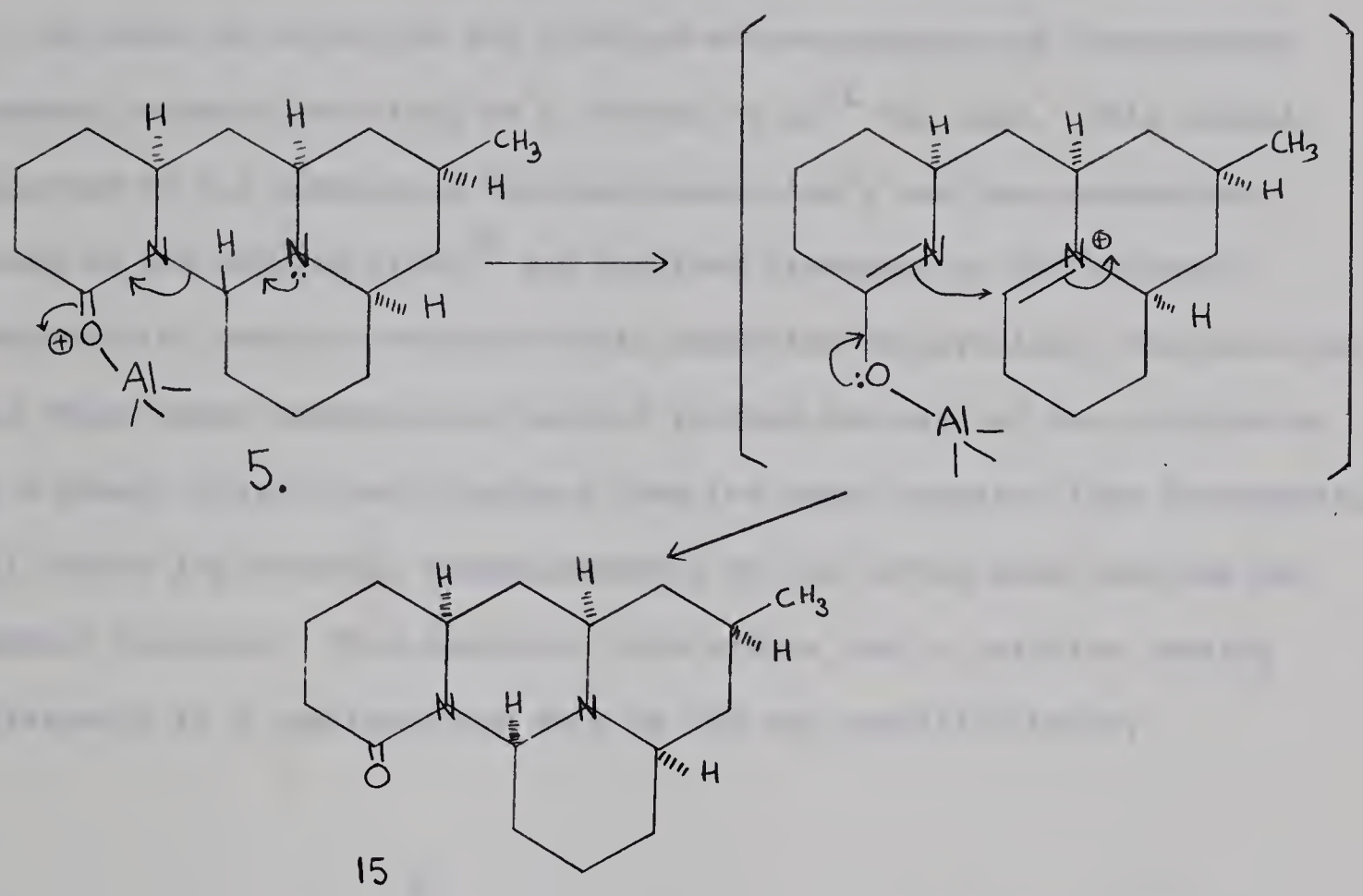
Lithium aluminum hydride reduction of both allocernuine (5) and epiallocernuine (15) yields the same product, dihydrodeoxyepiallocernuine, which is assigned structure 17 on the following grounds.



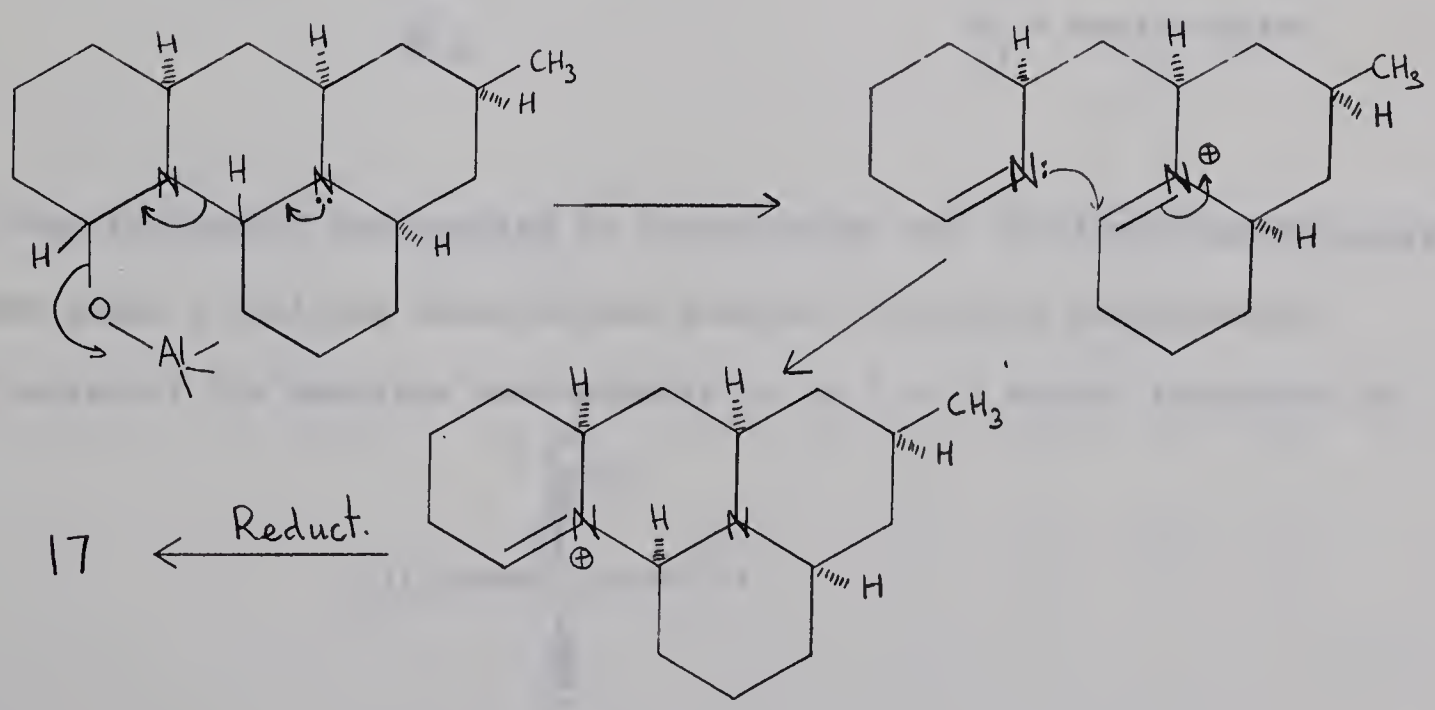
17.

In dihydrodeoxyepiallocernuine (17) both nitrogens are basic, and each one of them is flanked by three axial protons whose α-carbon-hydrogen bonds are trans-coplanar with the lone pair orbital of a nitrogen; this is an ideal situation for the development of Bohlmann bands and indeed 17 displays strong absorption in the 2700 - 2800 cm^{-1} region (see Fig.6). The isomerization of allocernuine to the epialloseries during the reduction could be due to a Lewis acid (aluminum hydride) catalyzed rearrangement (Scheme 1) prior to reduction or might also take place at the carbinol-amine stage in the reduction as indicated in Scheme 2.

Scheme 1

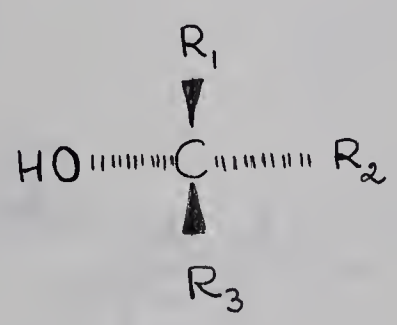


Scheme 2



The stereochemistry assigned to 17 has now been confirmed by synthesis[70].

In order to establish the absolute stereochemistry of lycocernuine a method recently described by A. Horeau et al.[71] was used. This method, described by its authors as "partial resolution", has been extensively tested in the steroid field[72] and involves treatment of the hydroxyl compound with ramecic α-phenylbutyric anhydride in pyridine. The empirical rule which these authors have derived is that the sign of the rotation of the α-phenyl butyric acid isolated from the above reaction (see Experimental) will define the absolute stereochemistry of the carbon atom carrying the hydroxyl function. This empirical rule states that a positive reading corresponds to a configuration such as the one specified below,

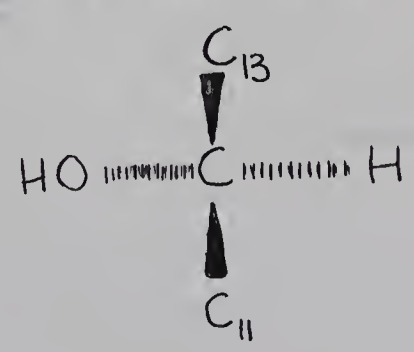


R_1 = large size

R_2 = small size

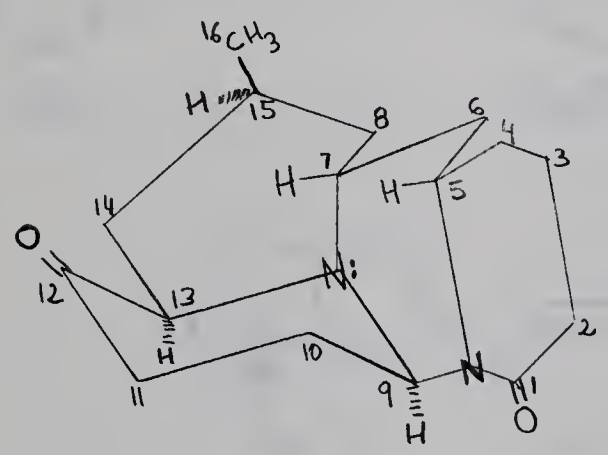
R_3 = medium size

When the method was applied to lycocernuine and to dihydrodeoxylycocernuine in both cases a positive rotation was observed for the α-phenylbutyric acid isolated; the absolute stereochemistry of C - 12 should therefore be:

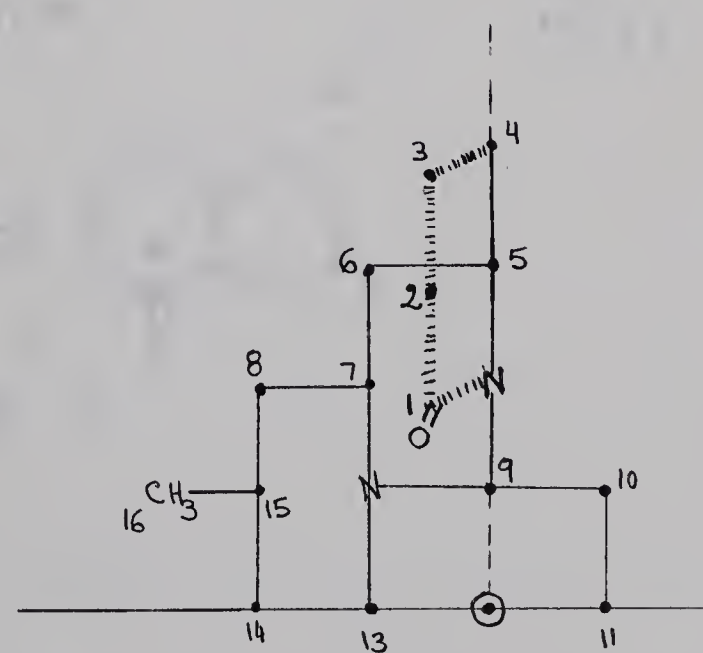


According to the rules laid down in the formulation of the "sequence rule"[73] C - 12 has the R-configuration in lycocernuine and 2c thus represents both the relative and the absolute stereochemistry of this natural compound.

Further confirmation of the absolute stereochemistry was obtained by applying the "Octant Rule" to dehydrolycocernuine. As can be seen in the octant diagram for 2c, the octant rule (provided it is applicable to 3-piperidones) predicts a positive Cotton effect curve for the ketone with the absolute stereochemistry shown. Dehydrolycocernuine does show a positive Cotton effect with extrema at 339 and 292 mμ and amplitude 6980°.



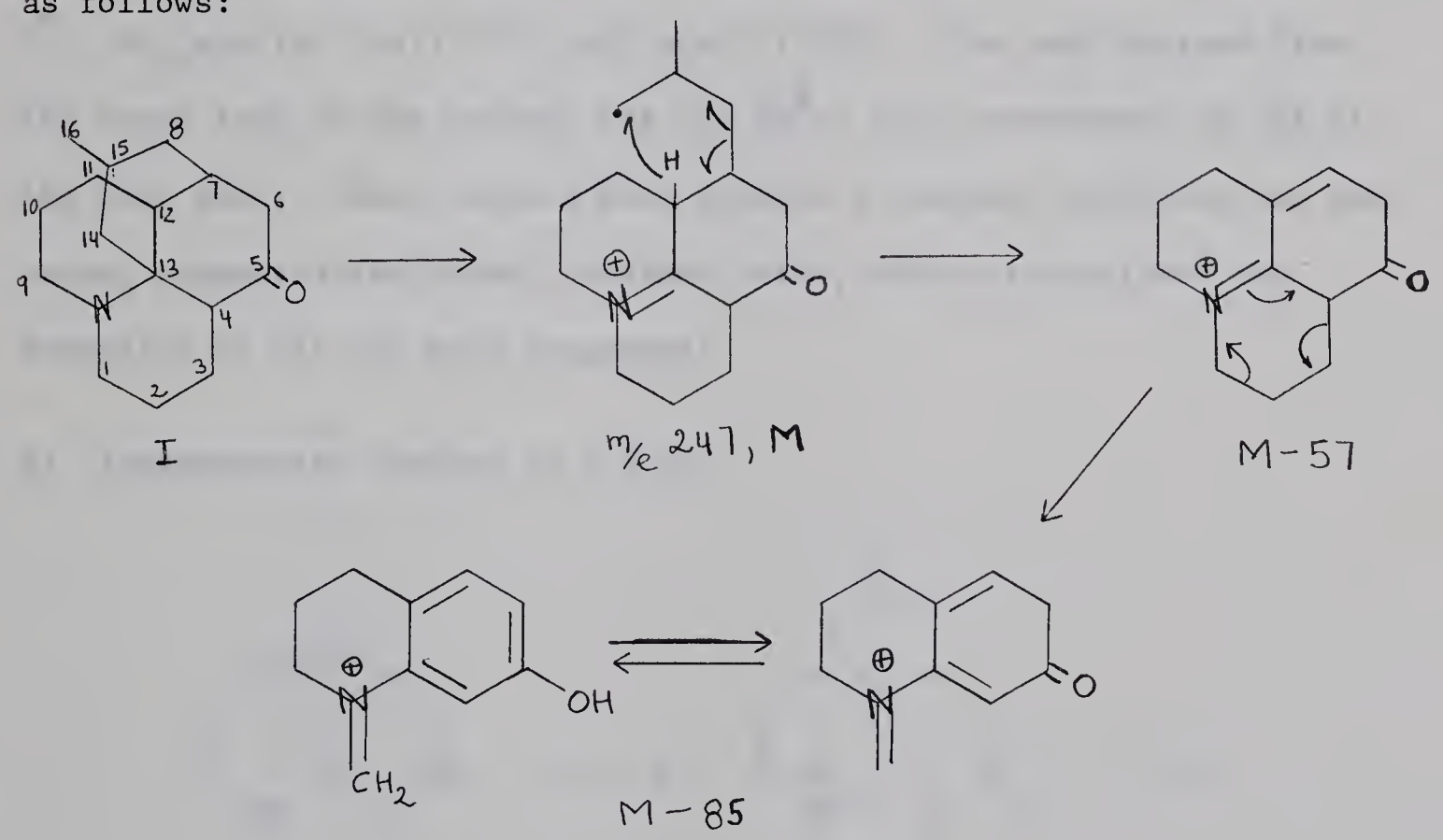
2c



Interpretation of the mass spectral data

It has been pointed out earlier (Discussion and Results) that the information available from the characteristic fragmentation pattern of both lycocernuine and cernuine served initially to exclude the possibility that these alkaloids contain a bridged-ring system such as the one encountered in lycopodine.

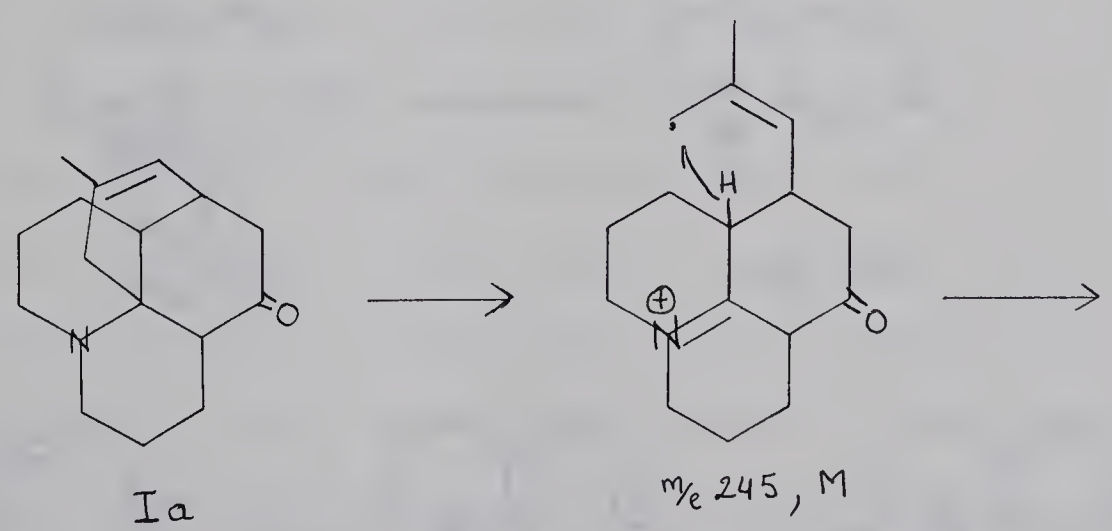
The principal mode of fragmentation of the Lycopodium alkaloids containing the usual bridging ring has been discussed by MacLean[34]; when applied to Lycopodine (I), the cracking pattern may be schematized as follows:



The main fragmentation mode involves the initial loss of the bridging system. In a lycopodine-type skeleton with a $\Delta^{11,12}$ unsaturation (acrifoline) or substituted at C - 12 (lycodoline (XI), the base peak

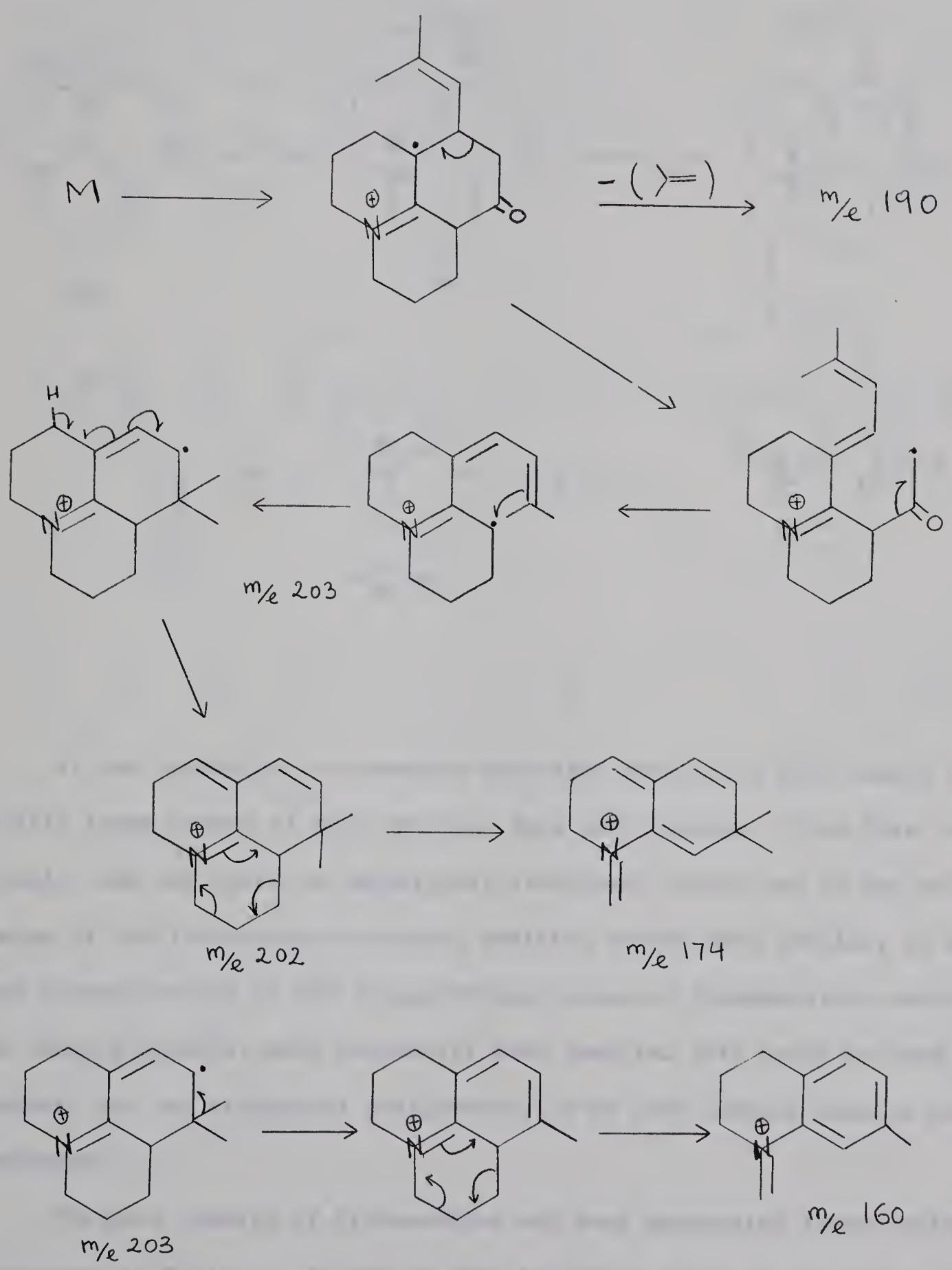
does not correspond to the loss of the bridging ring but, even in these cases, the fragment formed by loss of the bridging ring (no hydrogen can be abstracted from C - 12 as in lycopodine) still represents one of the main fragmentation routes. Ayer and Piers have also found that $\Delta^{8,15}$ dehydrolycopodine (Ia) also failed to exhibit loss of the bridging system as the main fragmentation mode. The mass spectrum of $\Delta^{8,15}$ dehydrolycopodine contained a parent peak at m/e 245 ($M^{\oplus}$), which was also the base peak of the spectrum. Other significant peaks correspond to: $M^{\oplus}$ - 15, m/e 230 (16)*; $M^{\oplus}$ - 42, m/e 203 (16); $M^{\oplus}$ - 43, m/e 202 (27); $M^{\oplus}$ - 55, m/e 190 (38); $M^{\oplus}$ - 57, m/e 188 (21); $M^{\oplus}$ - 71, m/e 174 (19); $M^{\oplus}$ - 85, m/e 160 (40); $M^{\oplus}$ - 108, m/e 137 (83). The peak derived from the usual loss of the bridge, m/e 190 ($M^{\oplus}$ - 55), corresponds to 38% of the base peak. These authors have offered a proposal involving two competing fragmentation paths, outlined below, which rationalises the formation of all the main fragments:

a) Fragmentation leading to m/e 160

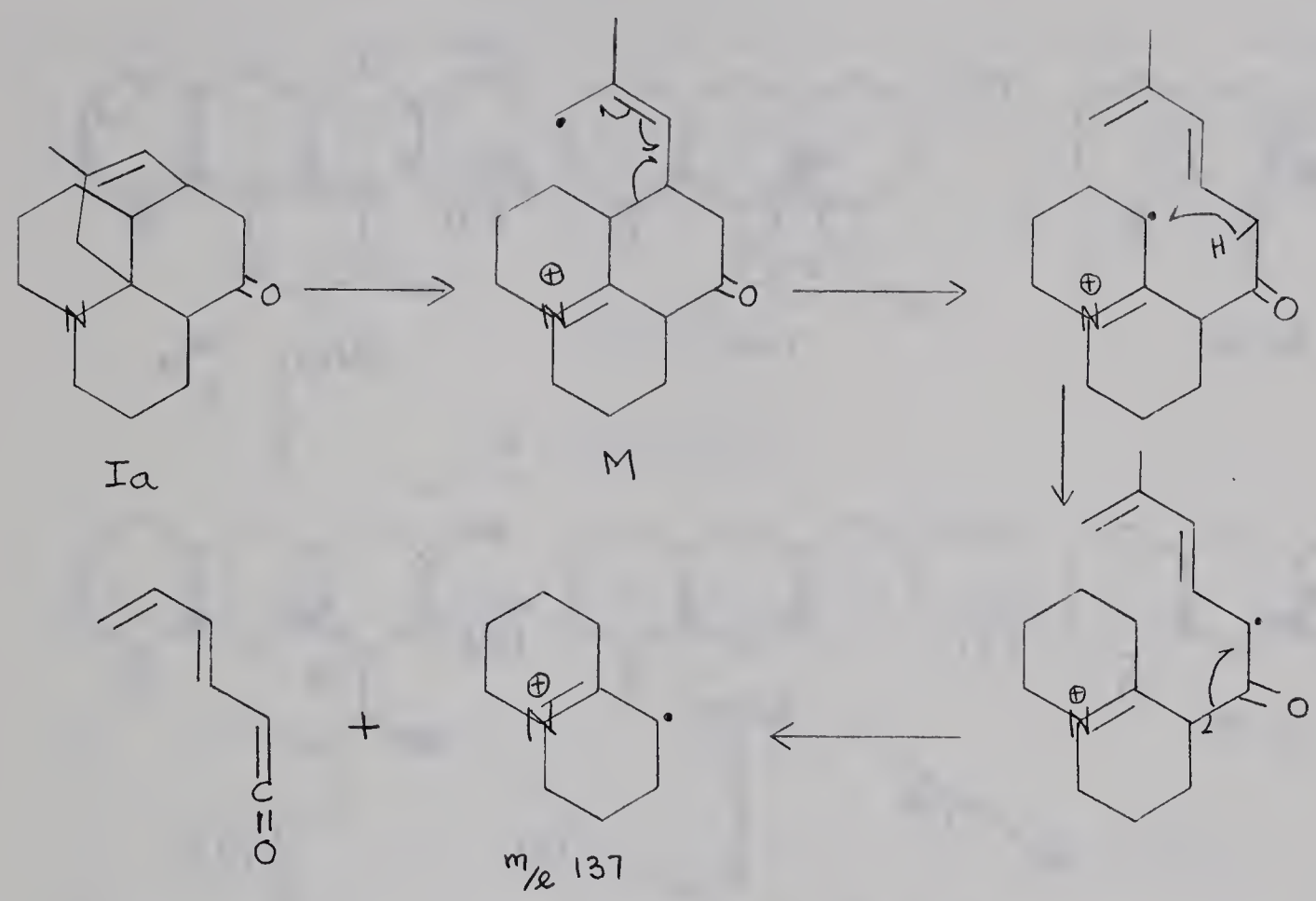


* Figures in brackets represent intensity of peak as a percentage of the base peak.

Fragmentation leading to m/e 160 (continued)



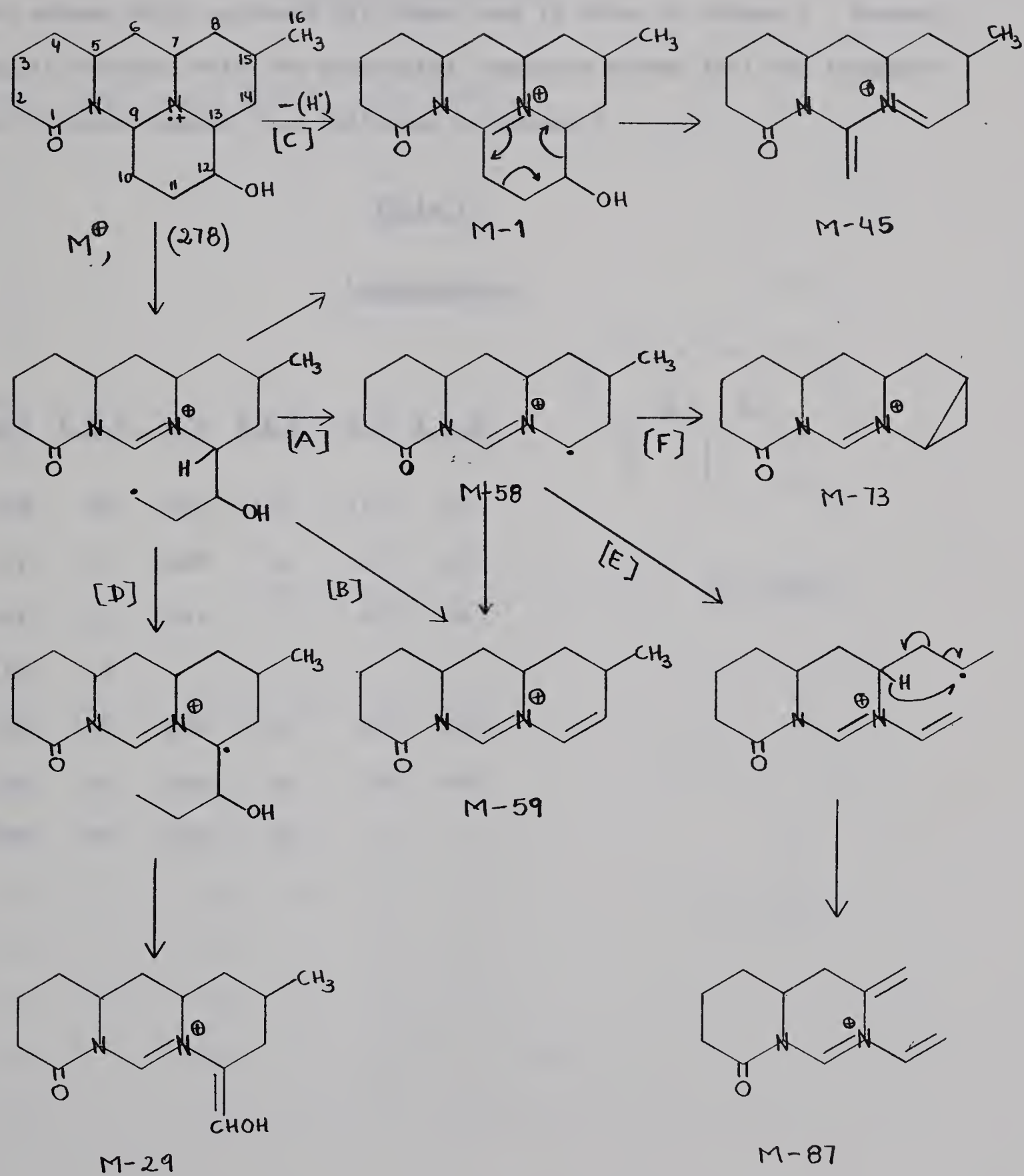
b) Fragmentation leading to m/e 137



In the course of the research described earlier in this thesis a fairly large amount of mass spectral data was obtained. This data, by itself, did not offer an unequivocal structural proof, but as the knowledge of the lycocernuine-cernuine skeleton became more precise, so did the interpretation of the characteristic modes of fragmentation encountered in these alkaloids, and, eventually mass spectral data could be used as support for our structural assignments, as we have already seen in some instances.

The mass spectra of lycocernuine and some deuterated lycocernuines are shown in Table 1. As may be seen important peaks in the high mass range

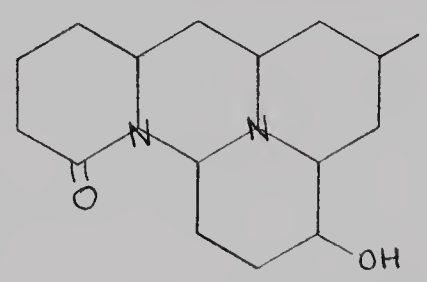
Scheme 1



are observed at m/e 261 (M - 17), 249 (M - 29), 233 (M - 45), 220 (M - 58), 219 (M - 59, base peak), 205 (M - 73) and 191 (M - 87). A simple fragmentation scheme which accounts for these ions is shown in Scheme 1. However, results obtained with the deuterated compounds showed that the fragmentation is more complex than indicated in Scheme 1.

Table I

Lycocernuine

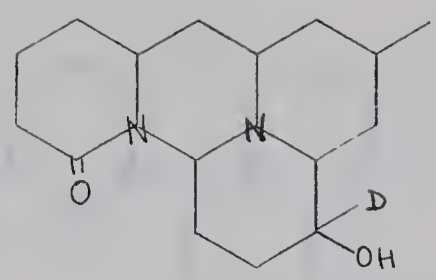


2b, M(278)

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
278	20	219	100	110	15
277	5	205	10	97	20
261	15	191	7	82	20
250	8				
249	20	166	22	69	22
233	10	165	35	55	50
220	60	152	30		

Table I (continued)

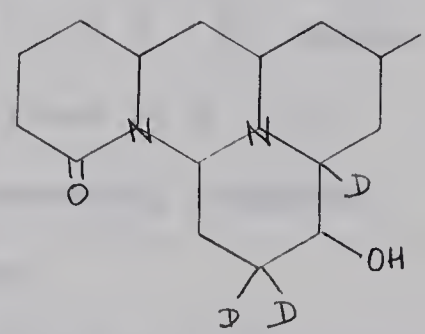
Lycocernuine-12-(d_1)



2f, M(279)

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
279	15	219	100	110	11
278	6	205	9	97	12
262	12	191	5	82	12
251	6				
250	18	166	20	69	7
234	5	165	42	55	19
220	62	152	32		

Lycocernuine, 11, 11, 13 (d_3)



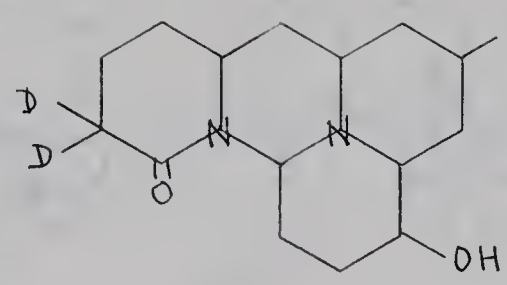
2g, M(281)

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
281	20	222	47	166	15
280	6	221	33	165	35
264	10	220	100	152	25
251	7	207	5	111	15
250	10	206	5	98	25
249	6	194	6	82,83	10 (each)
234	5	193	6	55	33

Table I (continued)

Lycocernuine-2,2-d_2

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
280	22	222	67	110	12
279	6	221	100	97	11
263	15	207	9	82	7
252	8	168	12	69	8
251	20	167	31	56	10
235	6	154	20		

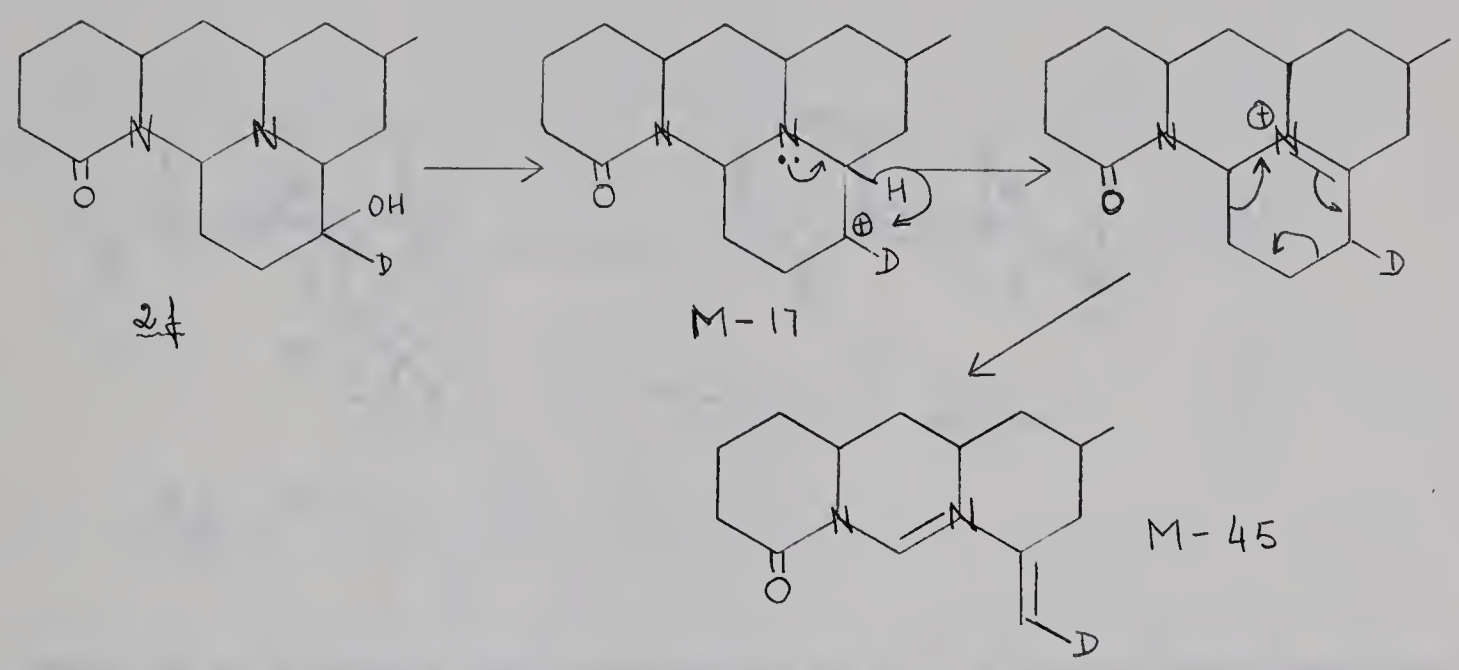


2h, M(280)

The mass spectrum of lycocernuine-12-(d_1), obtained by $NaBD_4$ reduction of dehydrolycocernuine, can be rationalized for the most part in terms of scheme 1. The peaks corresponding to M - 1, M - 17, M - 29, retain the deuterium atom as required by Scheme 1, while those at M - 58, M - 59, M - 73, possess the same mass as the corresponding fragments of lycocernuine, in agreement with the scheme above.

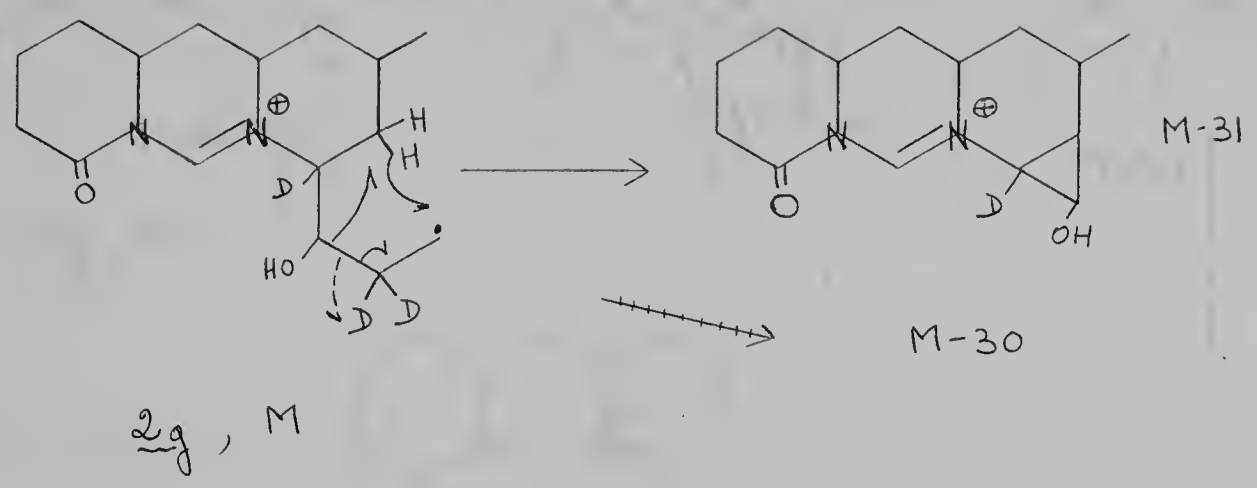
The process in Scheme 1 leading to the M - 45 fragment (path [C]) must however be revised since the expected fragment at m/e 233 is only 2% of the base peak, while the fragment at m/e 234 (retaining the deuterium atom) is 5% of the base peak. There must be a concurrent process by which the molecule losses 45 mass-units, one possibility is represented by the following scheme:

Scheme 2



The mass spectrum of lycocernuine - 11, 11, 13-(d_3) (obtained by acid catalyzed deuterium exchange of dehydrolycocernuine, the deuterated keto-compound being subsequently reduced with $NaBH_4$ in methanol-O-d) offered further insight into the actual fragmentation mechanism. The usual peaks at M - 1, M - 17 are present, but in the case of the M - 29 and M - 58 and M - 59 peaks the situation is more complex. The fragmentation written for M - 29 in Scheme 1 (path [D]) requires that in the present case all three deuterium atoms be lost, that is, a fragment at m/e 249 would be expected. Instead we find fragments at m/e 249 (6) and m/e 250 (10). Therefore in addition to the process written for M - 29 in Scheme 1 another pathway must be involved. A possible route is illustrated in Scheme 3.

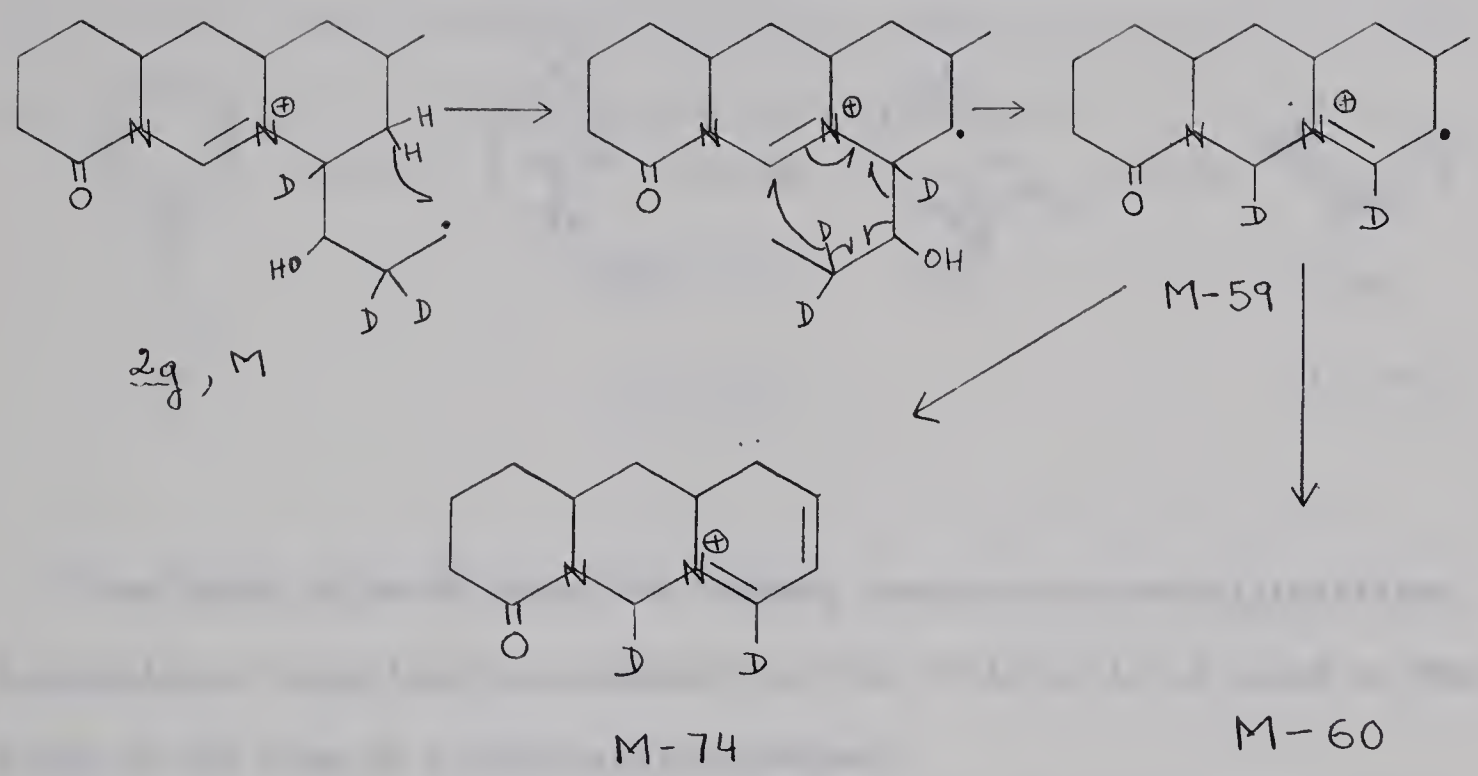
Scheme 3



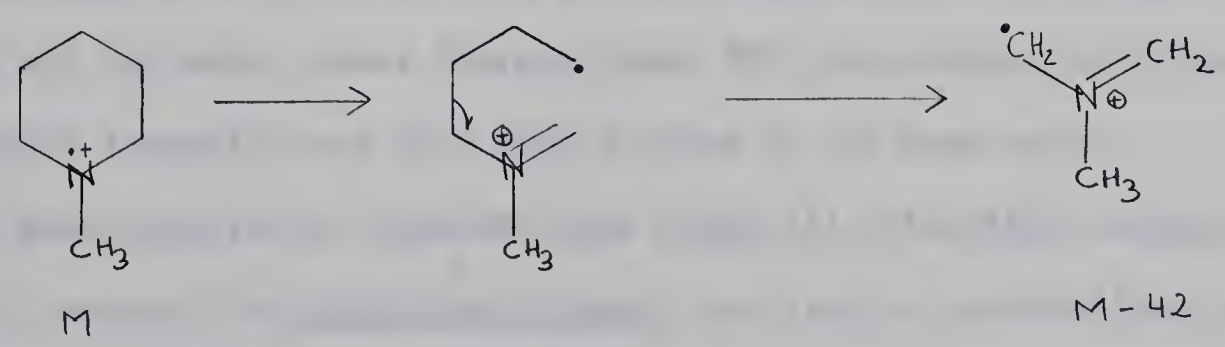
There is no evidence that abstraction at C - 14 occurs other than the fact that the radical-ion obtained after the abstraction could easily lose the methyl group; small fragments at M - 15 and M - 43 (15 + 28) are present in the mass spectrum of lycocernuine and lycocernuine-12(d_1); in the trideuteroderivative those fragments are at M - 15 and M - 45 respectively. It should also be noted that the M - 28 fragment present in lycocernuine and monodeuterolycocernuine appears at M - 30 in the spectrum of the trideutero compound in agreement with Schemes 1 or 3.

In the trideuterocompound the peak at m/e 219 is shifted to m/e 220, while the peak at m/e 220 is shifted in part to 221 and in part to 222 (ratio 3:4). The formation of this last fragment (m/e 222) implies a deuterium transfer from C - 11 to the ion. Scheme 4 accounts for this fragmentation:

Scheme 4

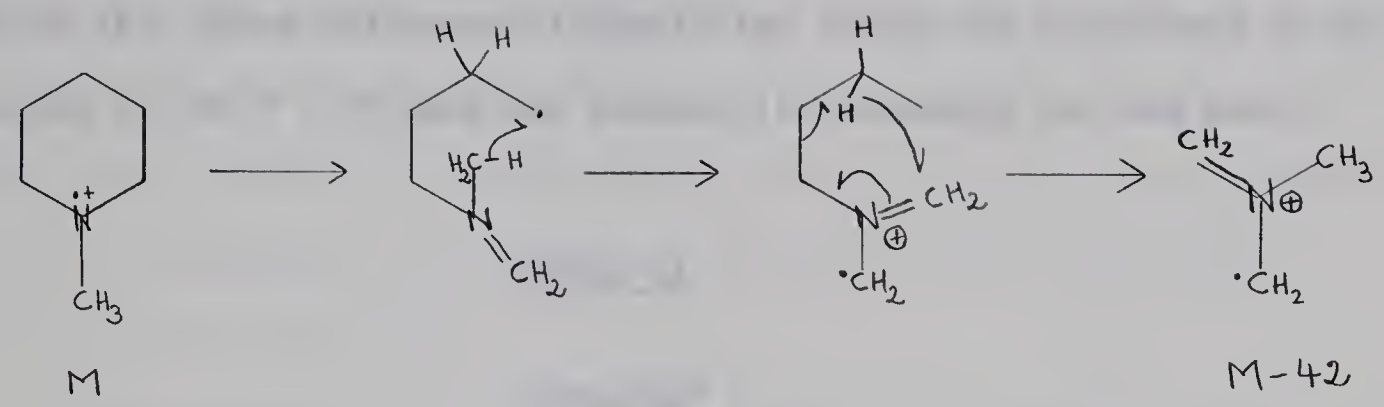


A process such as the one in Scheme 4, requiring a double hydrogen transfer has certain analogy in the fragmentation of N-methylpiperidine. Deuterium labelling experiments have shown[77] that the loss of 42 mass units from N-methylpiperidine, most easily rationalized as:



actually involve a double hydrogen transfer between the methyl group and

the methylenes:



The ratio in which these two schemes operate in N-methylpiperidine (according to labelling experiments) is 4:6. This ratio is close to that found in the case of trideuterolycocernuine.

Finally both radical-ions (M - 60) and (M - 59) formed in Schemes 1 (paths [A] and [B]) and 4 could lose 15 mass units to give fragments at M - 75 and M - 74. These two fragments (m/e 207 and m/e 206) are present in the mass spectrum of trideuterolycocernuine.

The schemes described above (1 - 4) explain (for the high-mass range) the fragmentation pattern of a deuterated derivative of lycocernuine such as lycocernuine-2,2,-d_2 (see Table 1). The mass spectrum of this compound exhibits all the major peaks (down to mass 207) encountered in lycocernuine with similar intensity and with each shifted by two mass units.

The mass spectrum of cernuine (see Table II), the other natural occurring compound in Lycopodium cernuum, can also be rationalized on the basis of the proposed schemes (1 - 4).

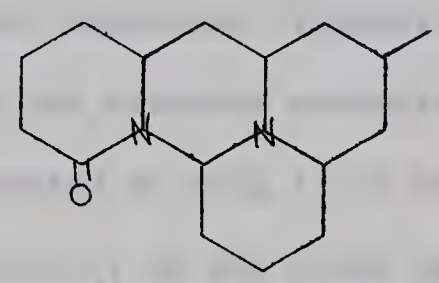
Loss of carbon C - 10, C - 11, and C - 12 (Schemes 1 and 4) would lead to ions at M - 42 (m/e 220) and M - 43 (m/e 219). The base peak in

cernuine is at M - 29 (m/e 233). It is possible to arrive at this fragment by three different routes depicted in Schemes 1 (path [D]), 2, and 3. The fact that three different fragmentation routes can contribute to the formation of the M - 29 peak may explain its intensity in this case.

Table II

Cernuine*

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
262	40	220	65	164	15
261	11	219	50	150	15
247	15	205	20	136	12
234	22	191	20	122	12
233	100	178	15	110	11



2a, M(262)

Also, the hydroxyl group in lycocernuine may help to facilitate the formation of the M - 58, M - 59 fragments (Scheme 1) by stabilizing the radical at C - 12 formed during such a process.

Further evidence for some of these processes is supplied by the presence of metastable ions at m/e 191.5, 183.4 and 167.5 in the cernuine

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* The mass spectra of allo- and epiallocernuine are almost identical to that of cernuine.

[illegible]

[illegible]

[illegible]

system. Thus, the calculated metastable peak for the fragmentation 262 → 219 is 183, found 183.4; the proposed transformation of the m/e 220 ion into m/e 205 (Scheme 4) is supported by the appearance of a metastable peak at 191.5, (calculated for 220 → 205, 191.1), and the appearance of a metastable peak at 167.5 suggests that the m/e 220 radical-ion (Scheme 1) may lose 29 man-units to give rise to the m/e 191 peak (calculated 165.4) (Scheme 7, path [E]).

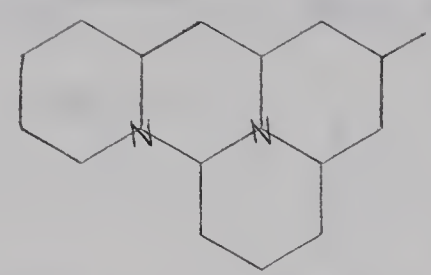
All the processes referred to in schemes 1 - 4, involve fragmentation of ring D only; in fact they account for the most important fragments formed on electron impact with the lycocernuine and cernuine molecules. Accordingly the mass spectra of compounds deuterated in ring A, as we have already seen in the case of lycocernuine-2,2-d_2, do not offer any new feature, all the fragments rationalized in Schemes 1 - 4 being consistently shifted by two mass units. It can be predicted that the same behaviour will be encountered with the dihydrodeoxyderivatives of both lycocernuine and cernuine in which the lactam carbonyl of ring A has been converted into a methylene group. Comparison of Tables III and IV with Tables I and II shows that this is the case. All the fragments, in the high-mass range of the mass spectrum of dihydrodeoxycernuine (Table III) predicted by Schemes 1 - 4 are present except that they are shifted by 14 mass units with respect to those of cernuine (Table II).

The same is true for dihydrodeoxylycocernuine (Table IV) when compared with lycocernuine (Table I).

Table III

Dihydrodeoxycernuine

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
248	55	205	80	136	66
		191	22		
247	35	177	25	122	12
233	19	164	24	110	15
220	26	151	39	97	22
219	100	150	49	84	39
206	89	137	23	55	38

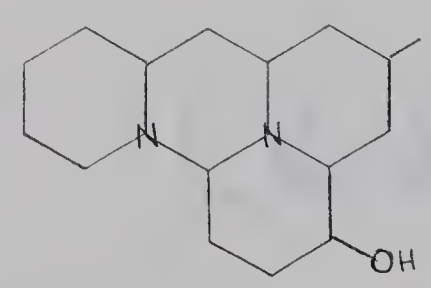


20, M(248)

Table IV

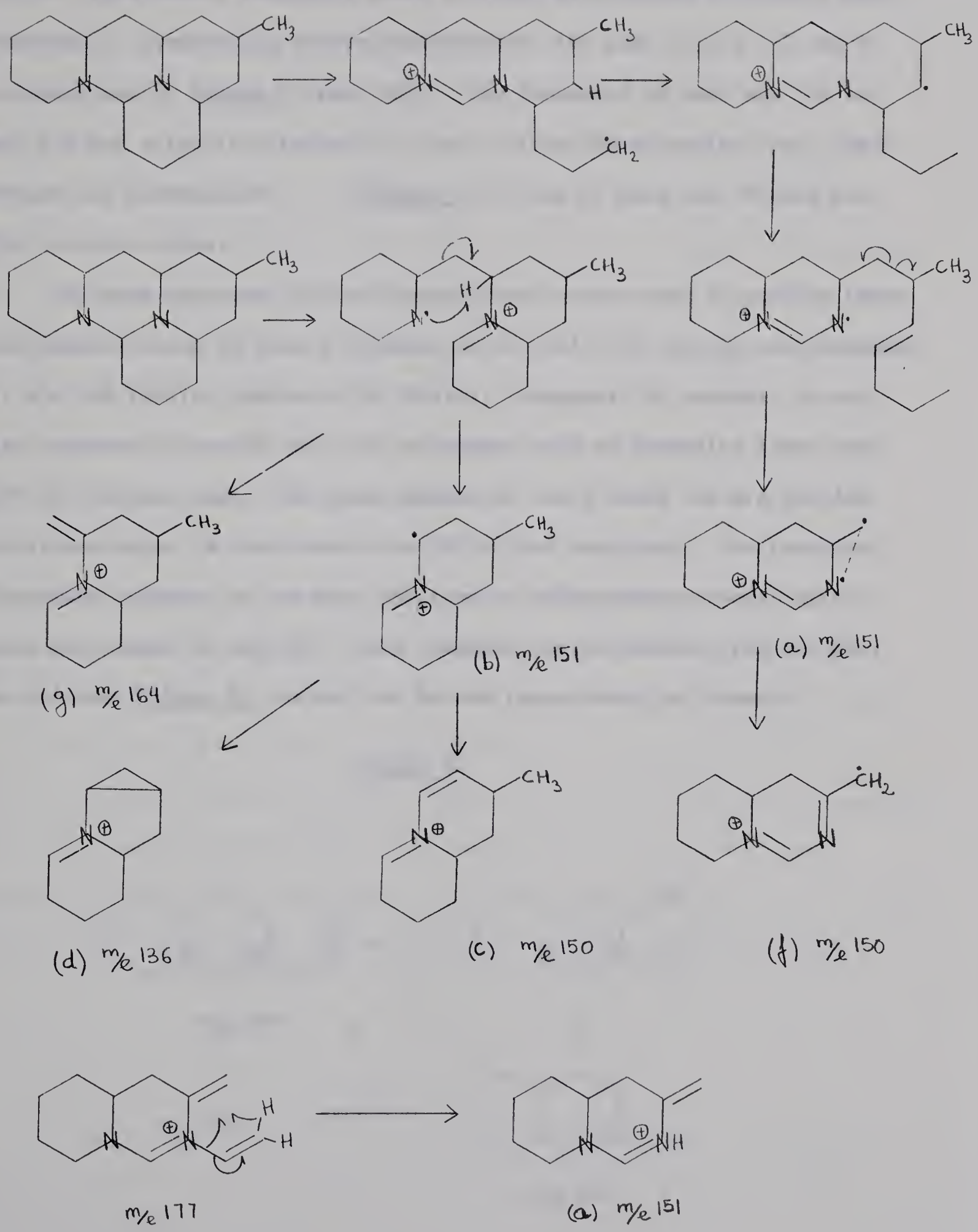
Dihydrodeoxylycocernuine

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
246	12	191	7	124	20
263	18	177	5	110	16
247	16	166	20	98	20
236	10				
235	17				
219	10	152	24	84	22
206	35	151	24	69	20
205	100	138	20	55	25



12a, M(264)

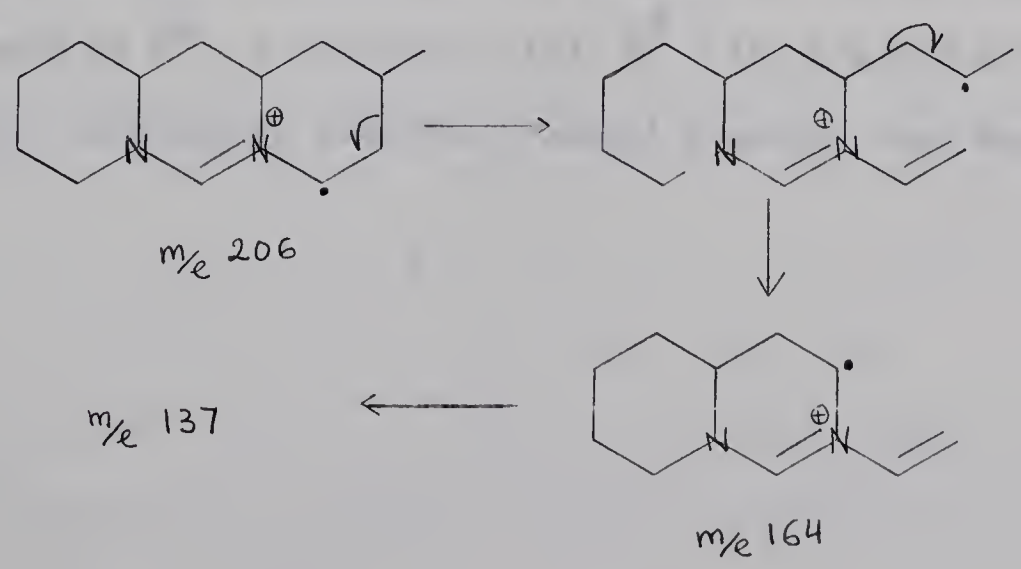
Scheme 5



Thus far only the peaks in the high mass range have been considered. In the dihydrodeoxy compounds peaks at lower mass become relatively more important. Considering dihydrodeoxycernuine the peak at m/e 177 can be rationalized by Scheme 1 (path [E]). The fragments of mass m/e 151 and m/e 150 may arise from the m/e 177 ion or from the molecular ion. These routes are schematized (Scheme 5). Ions of mass m/e 164 and m/e 136 are also shown.

The mass spectrum of dihydrodeoxylycocernuine seems to confirm these assignments since we find a fragment at m/e 151 (39) (ion a) and fragments at m/e 166 (20)(c), and m/e 152 (24)(d). Fragment (b) probably is not very important since the m/e 167 is present with an intensity lower than 10% of the base peak. The same applies to ion f since the m/e 150 ion in lycocernuine is also lower than 10% of the base peak. The remaining important fragment in the mass spectrum of dihydrodeoxycernuine within this mass range is m/e 164. This fragment can be derived from m/e 206, as follows (Scheme 6), as well as by the route shown in Scheme 5.

Scheme 6



In dihydrodeoxylycocernuine we find small fragments (less than 10% of base peak) at m/e 164 and m/e 180, corresponding to those two different fragmentation modes.

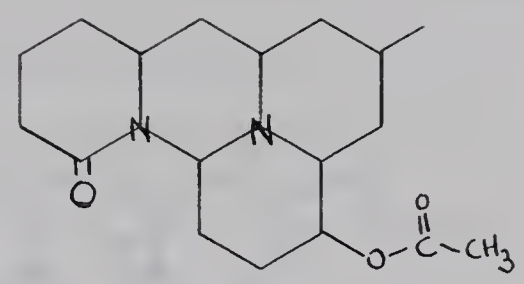
An accurate mass determination on fragments m/e 150 and m/e 164 reveals that in fact these peaks are doublets containing one and two nitrogens as required if both Schemes 5 and 6 are operative. The most intense part of the doublet at m/e 150 corresponds to $C_{10}H_{16}N$ in agreement with our qualitative deductions. The m/e 164 fragment is made up of ions with composition $C_{11}H_{18}N$ (Scheme 5, most intense) and $C_{10}H_{16}N_2$ (Scheme 6).

Metastables peaks at m/e 177.2, 171.4 and 109.2 in the mass spectrum of dihydrodeoxycernuine offer support for the occurrence of processes: 206 → 191 (Scheme 1, path [F]), calculated: 177.0; 248 → 206 (Scheme 1, path [A]), calcd. 171.1 and 248 → 164 (Scheme 5), calcd. 108.6.

The mass spectrum of simple derivatives of lycocernuine such as O-acetyllycocernuine (Table V) can be interpreted on the basis of the schemes outlined above except for certain fragments the formation of which can be understood by taking into consideration the peculiarities of the newly introduced functional group. Thus in the case of O-acetyllycocernuine, apart from a peak corresponding to the molecular ion at m/e 320 (15), fragments such as $M^{\oplus}$ - 43, m/e 277 (15); $M^{\oplus}$ - 58, m/e 262 (20) and $M^{\oplus}$ - 59, m/e 261 (90), originating from the O-acetyl function, are encountered.

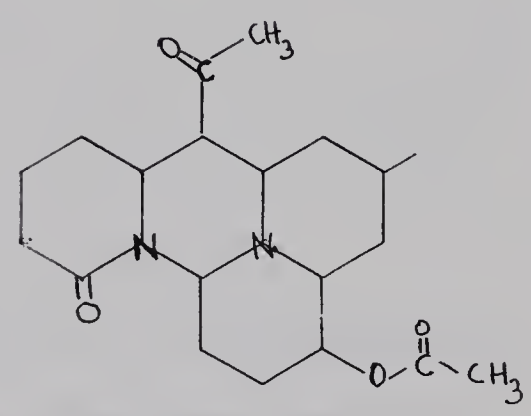
Table V

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
320	15	249	50	134	7
305	7	220	35	110	7
292	5	219	100	98	15
277	15	205	6	82	10
262	20	162	15	69	15
261	90	149	20	55	35



21, M(320)

m/e	% B.P.	m/e	% B.P	m/e	% B.P.
362	8	262	20	166	15
320	15	261	100	148	20
319	80	259	16	134	20
304	12	219	20	98	25
303	64	204	8	80	15
291	40	194	7	69	20

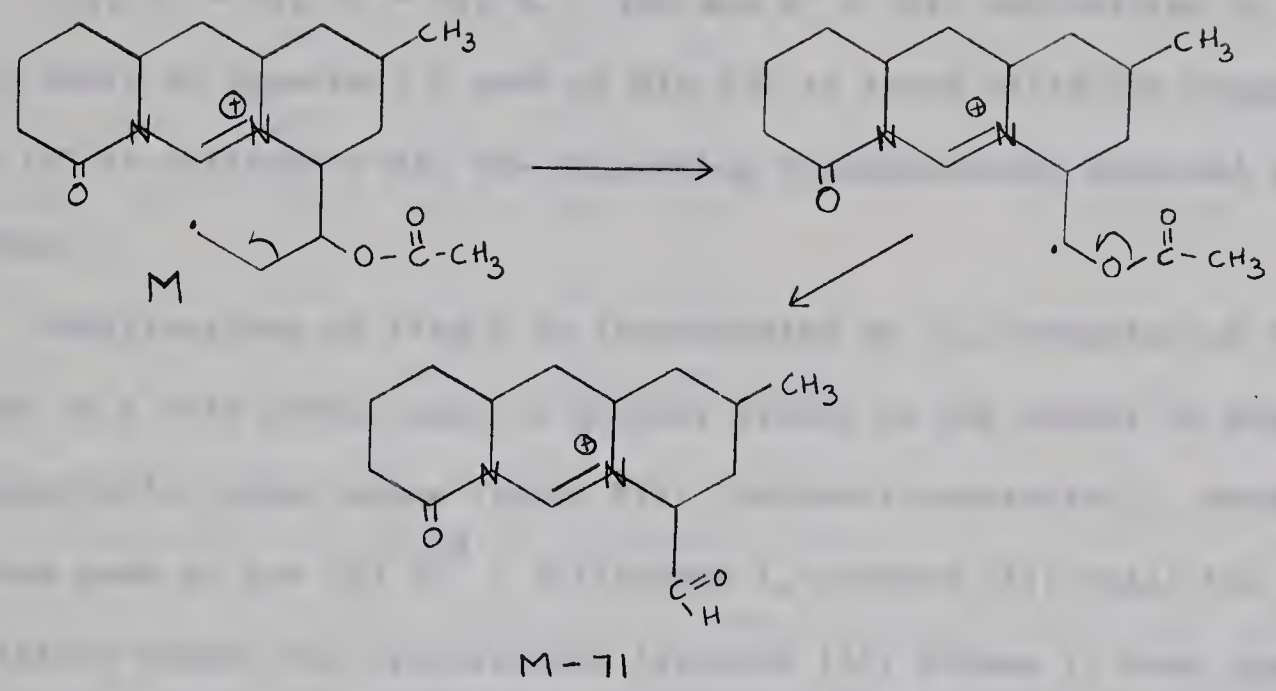


18, M(362)

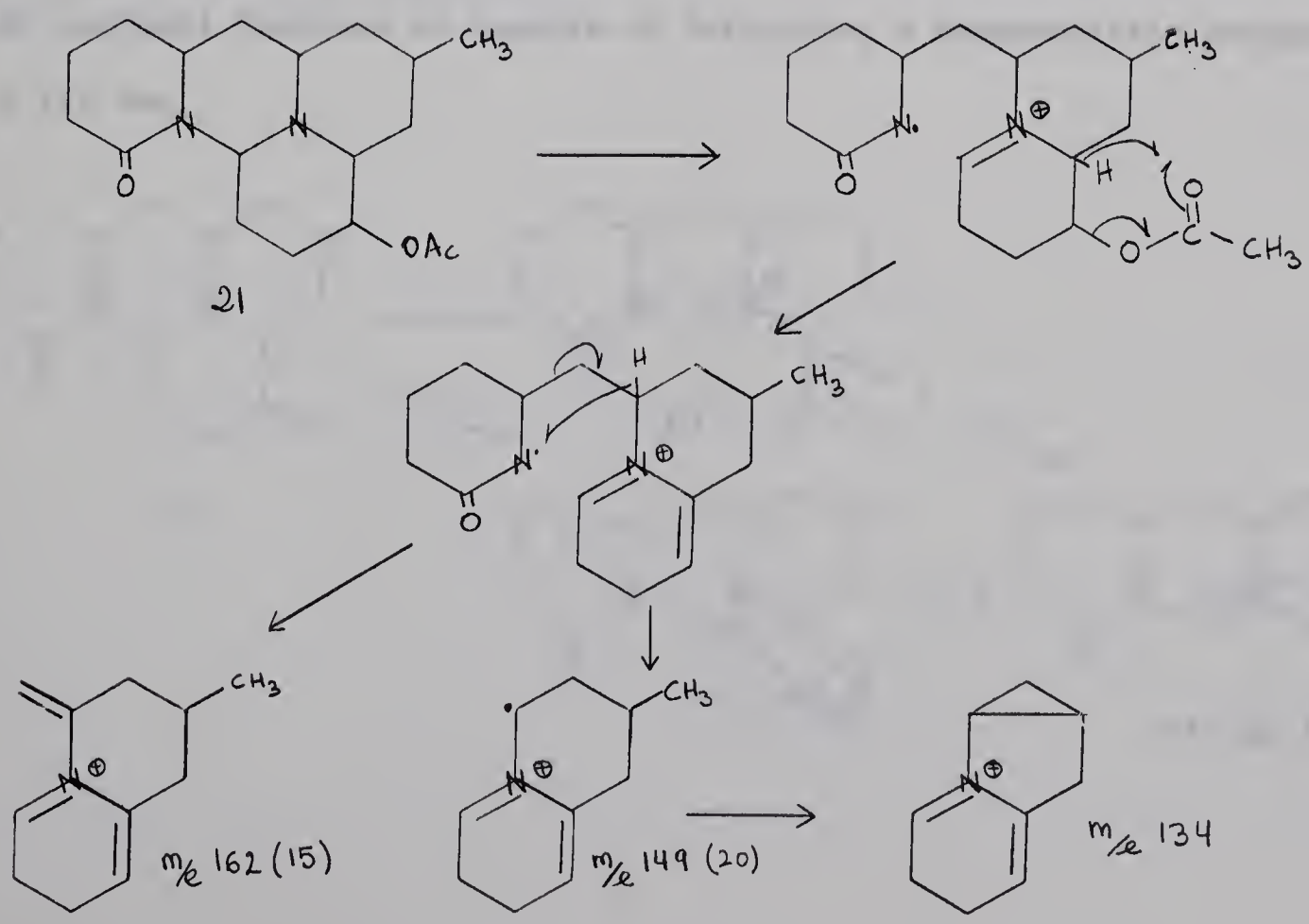
Ions corresponding to $M^{\oplus}$ - 100, m/e 220 (35) and $M^{\oplus}$ - 101, m/e 219 (100) are easily derived by processes summarized in Scheme 1; other prominent fragments such as the one at m/e 249 (50), ($M^{\oplus}$ - 71), must be due to the consecutive losses of fragments (43 + 28) or (42 + 29).

Fragmentations (similar to processes in Scheme 5) must account also for the appearance of three medium intensity fragments at m/e 162 (15); m/e 149 (20) and m/e 134 (7),

Scheme 7



Scheme 7'



In agreement with these schemes 18, obtained by acetylation of the oxazolidine 10 (see Discussion and Results, p. 55) followed by hydrogenation, exhibits a mass spectrum (Table V) the main fragments of which ($M^{\oplus}$ - 43, $M^{\oplus}$ - 59, $M^{\oplus}$ - 71, $M^{\oplus}$ - 100 and $M^{\oplus}$ - 101) are shifted by 42 mass units as expected; a peak at m/e 134 is found while the fragment at m/e 162 is shifted to m/e 204 supporting fragmentations depicted in Scheme 7.

Modifications of ring D in lycocernuine (e. g. oxidation of the hydroxyl group to a keto group) exert a greater effect on the manner in which the fragmentation takes place (Table VI). Dehydrolycocernuine 7, exhibits a base peak at m/e 220 ($M^{\oplus}$ - 56)(Scheme 1, process [A]) while the fragmentation scheme for lycocernuine (process [B], Scheme I) when applied to the dehydroderivative would lead to a $M^{\oplus}$ - 57 ion at m/e 219 which appears here with an intensity of only 60% of the base peak.

The reason for this reversal can be understood by considering that the carbonyl function is capable of initiating a fragmentation process of its own,

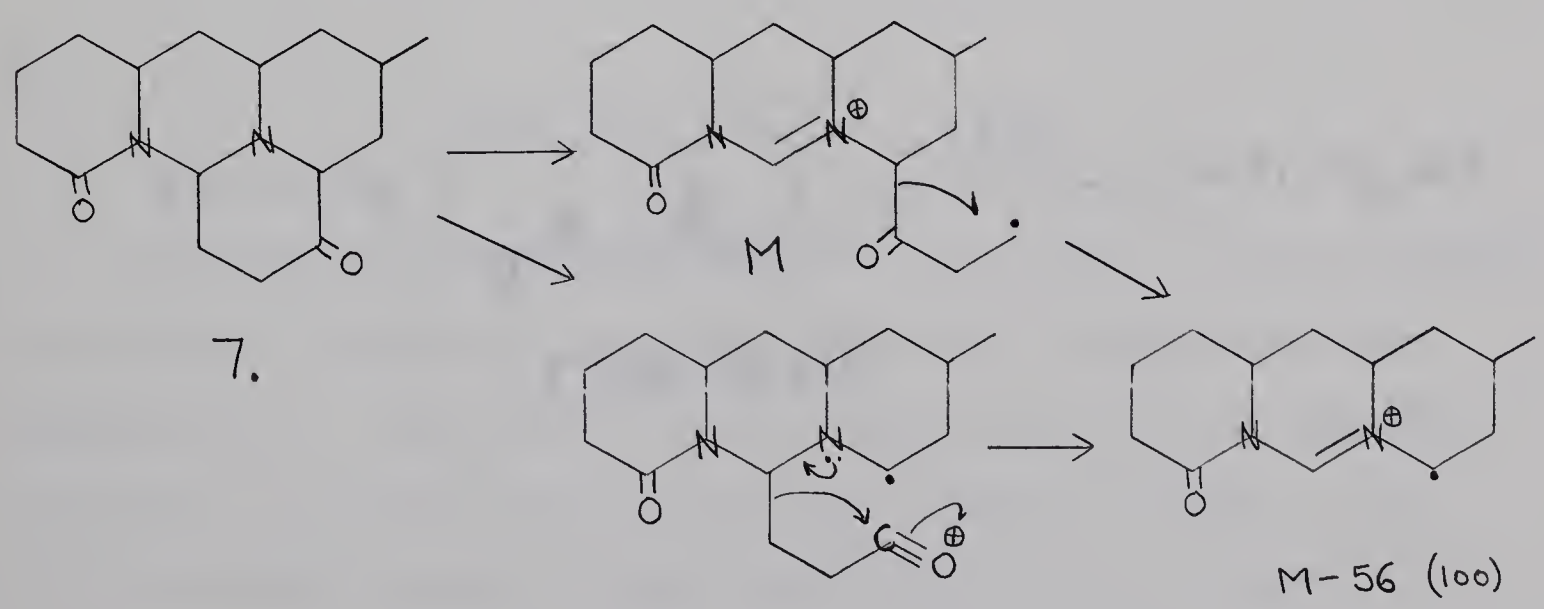
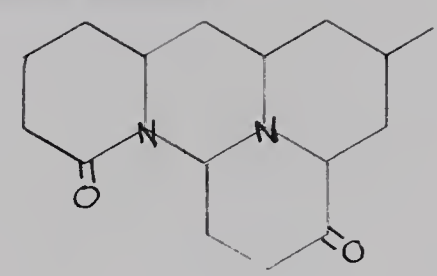


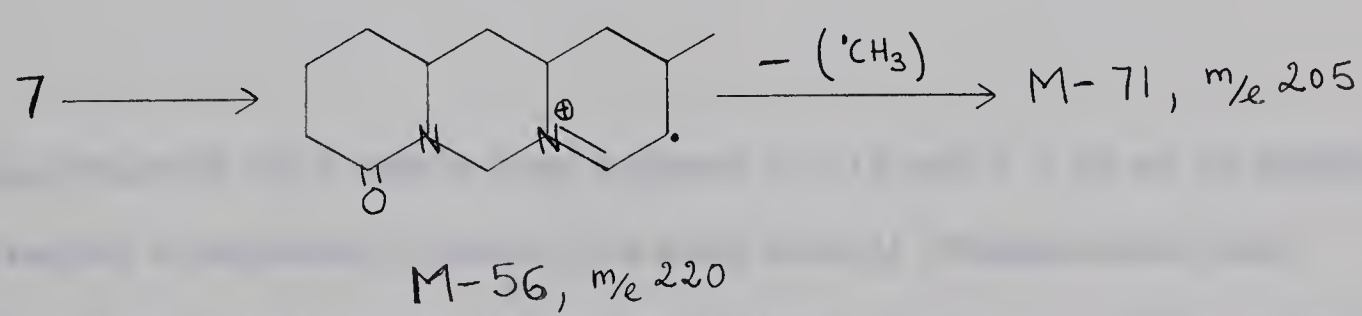
Table VI

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
276	7	192	15	149	15
249	45	191	12	137	15
220	100	178	30	110	25
219	60	164	20	96	15
205	40	152	17	82	18

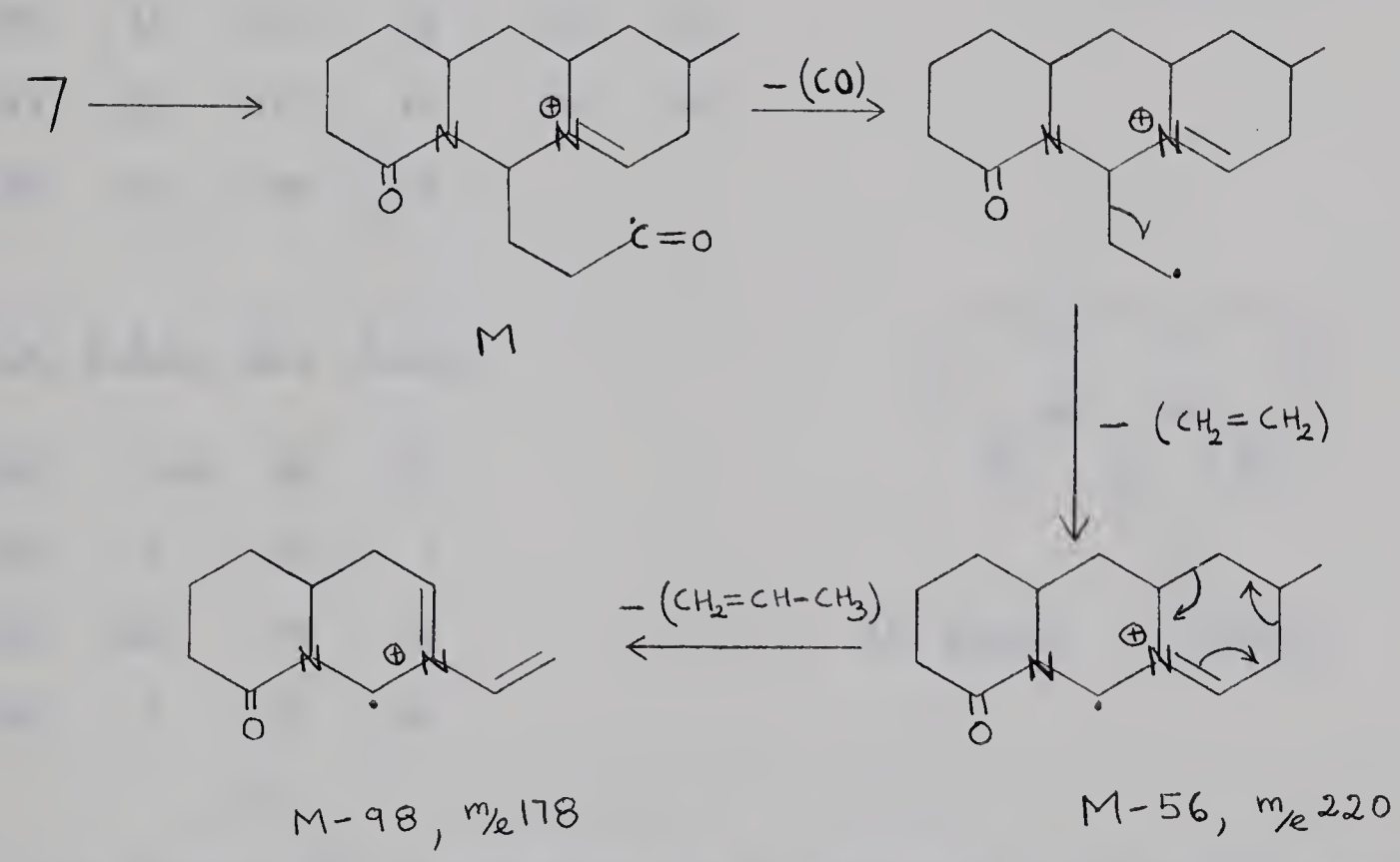


7, M(276)

or facilitating the homolytic cleavage of the C - 12 - C - 13 bond in process [A], which thus prevails; it seems also quite possible that the hydrogen-abstraction processes implied in Scheme 4 would be facilitated in the case of dehydrolycocernuine; a strong peak at m/e 205 (40) obtained by loss of the methyl group from the base-peak ion of Scheme 4, is in agreement with this assumption:



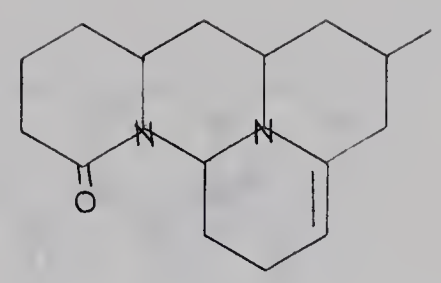
Other peculiar features of the mass spectrum of dehydrolycocernuine may be attributed to the influence of the carbonyl group:



Introduction of a double bond between C - 12 and C - 13 as in anhydrolycocernuine 4 completely disrupt the main mode of fragmentation described earlier. This result is not surprising since the main fragments obtained in previous schemes always involve cleavage of bonds of ring D. Loss of these carbons in anhydrolycocernuine is not as favored and the fragmentation pattern is drastically altered in this compound (Table VII).

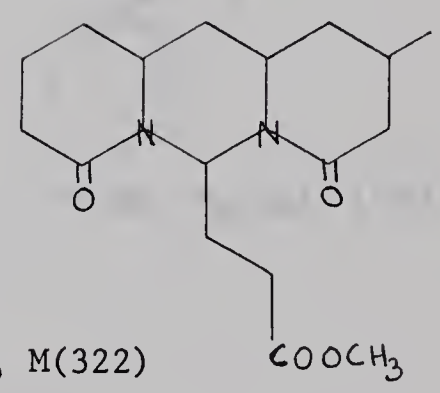
Table VII

m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
260	50	149	100	93	25
259	62	148	25	80	13
245	36	134	22	69	15
232	12	120	18	67	15
217	20	107	14	55	50
162	45	98	14		



4, M(260)

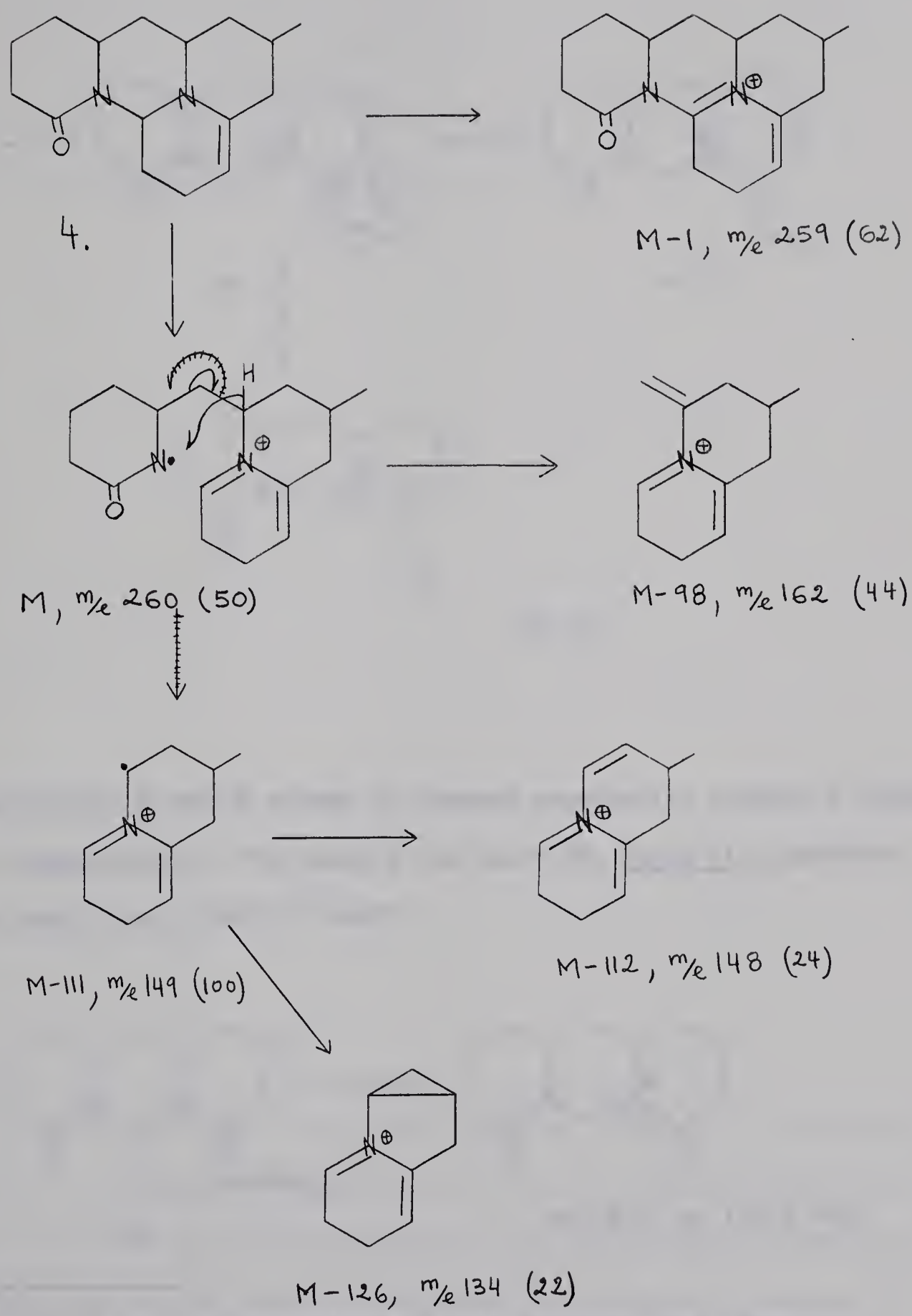
m/e	% B.P.	m/e	% B.P.
322	0.6	98	12
291	6	82	5
235	100	69	15
207	6	55	18



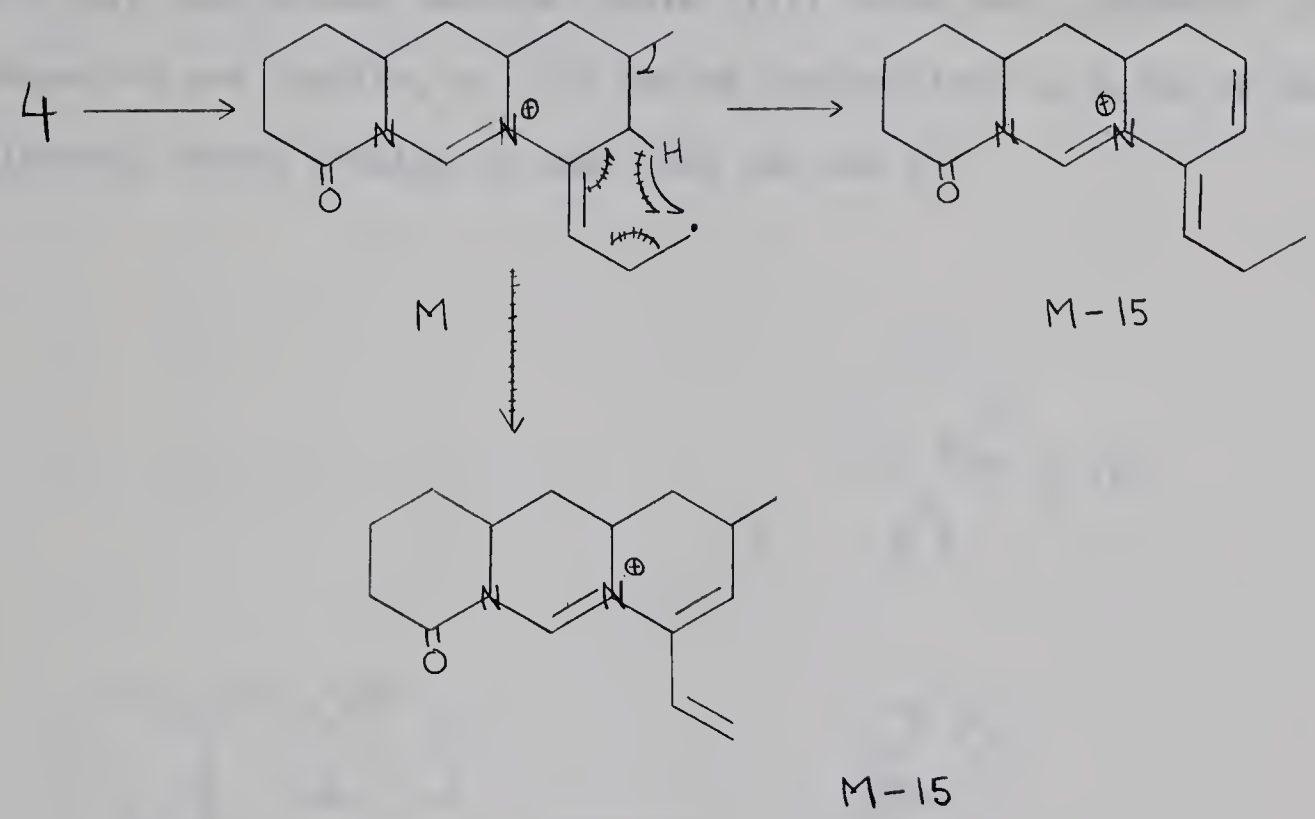
19, M(322)

This compound (4) is the first one to exhibit a fairly intense parent peak [m/e 260, (50)] and even stronger $M^{\oplus}$ - 1 peak at m/e 259 (62). Other fragments are: $M^{\oplus}$ - 15, m/e 245 (36); $M^{\oplus}$ - 98, m/e 162 (44); $M^{\oplus}$ - 111, m/e 149 (100); $M^{\oplus}$ - 112, m/e 148 (24) and $M^{\oplus}$ - 126, m/e 134 (22).

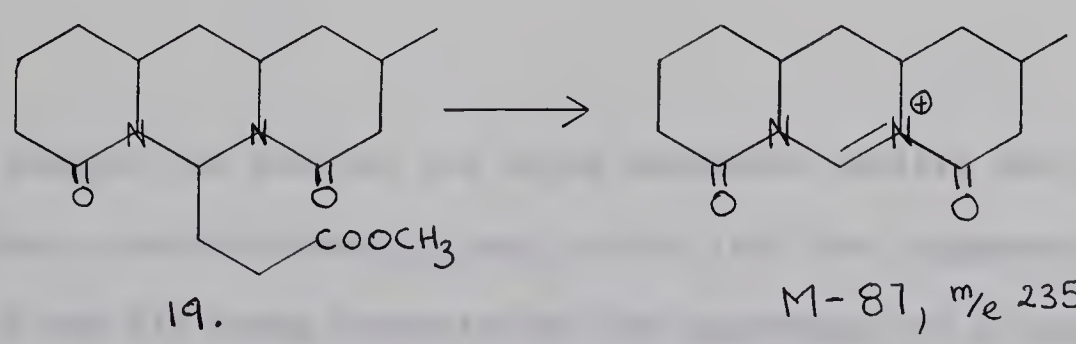
The following processes could account for the formation of those fragments:


4.
M-1, m/e 259 (62)
M, m/e 260 (50)
M-98, m/e 162 (44)
M-111, m/e 149 (100)
M-112, m/e 148 (24)
M-126, m/e 134 (22)

The $M^{\oplus}$ - 15 fragment may originate from the molecular ion as indicated:



Derivatives in which a ring is cleaved necessarily undergo a different type of fragmentation. For example the ester 19, Table VII, exhibits its base peak at m/e 235* ($M^{\oplus}$ - 87)



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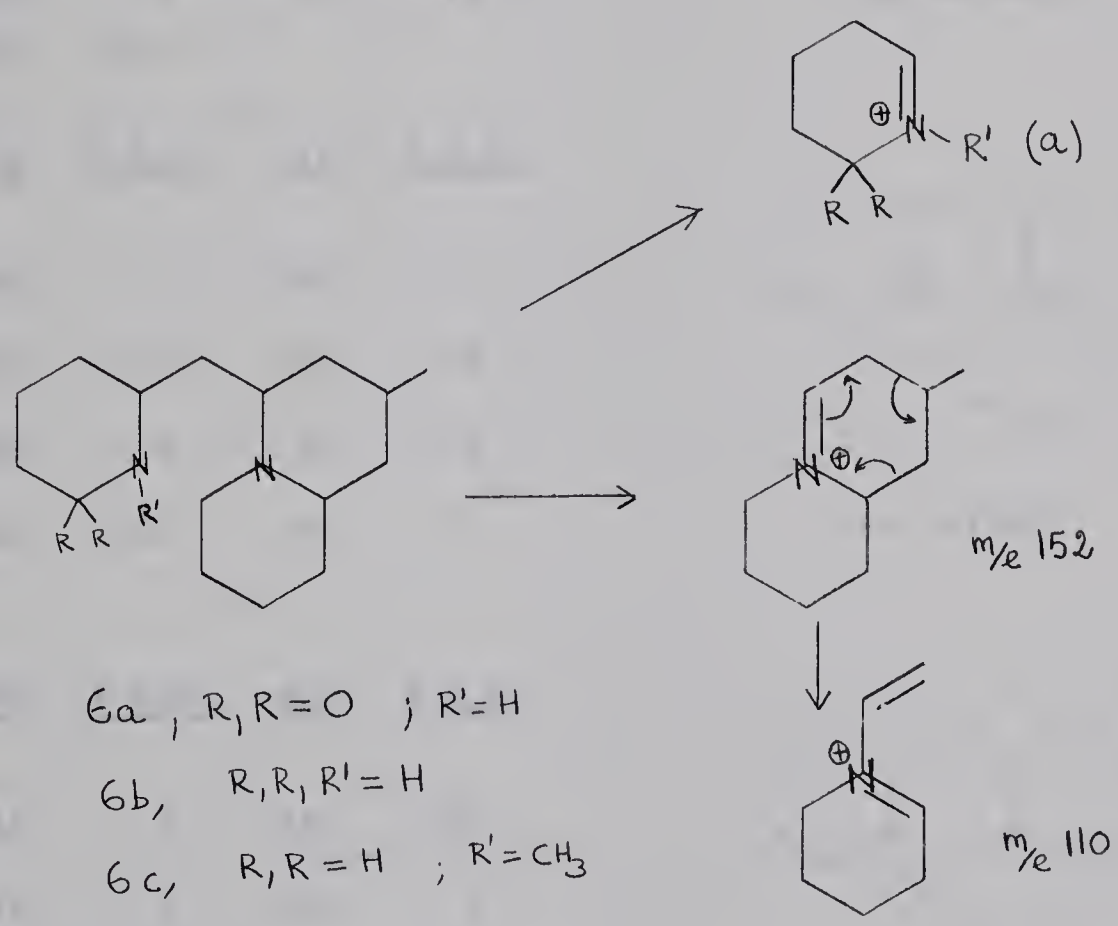
* Accurate mass determination of this fragment (m/e, measured 235.1447; calculated, for $C_{13}H_{19}N_2O_2$, 235.1443) supports the assignment.

Most of the other fragments present in the spectrum of 19 have intensities less than 10% of the base peak.

Derivatives such as 6a, 6b, and 6c, in which ring B is cleaved, give very simple mass spectra (Table VIII) whose main fragments (see Discussion and Results, p. 44) can be rationalized in terms of the following scheme leading to ions (a), (b) and (c)

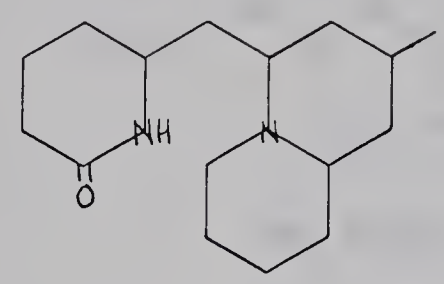


As pointed out earlier the three compounds exhibit the base peak at m/e 152 and a medium strength peak at m/e 110, the fragmentation from m/e 152 → m/e 110 being supported by the appearance of a metastable peak at m/e 80.1 (calculated for 152 - 110, 79.6) in all three spectra. Ion (a) of mass 98 in 6a and 6c, is much stronger in this last compound; in

6b it appears at m/e 84 almost with same intensity as in 6c (50).

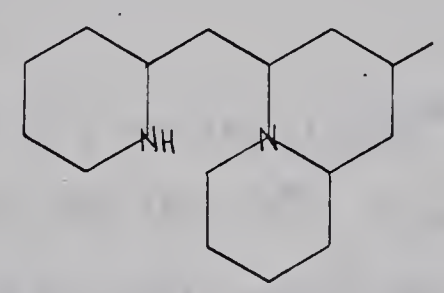
Table VIII

m/e	% B.P.	m/e	% B.P.
264	3	110	16
233	4	84	8
166	6	55	18
152	100		



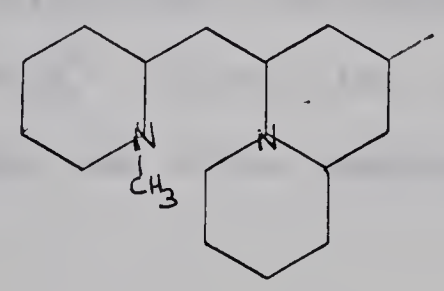
6a, M(264)

m/e	% B.P.	m/e	% B.P.
250	5	124	11
205	13	110	18
166	8	97	16
152	100	84	25



6b, M(250)

m/e	% B.P.	m/e	% B.P.
264	9	152	100
249	6	124	6
178	8	110	18
166	10	97	50

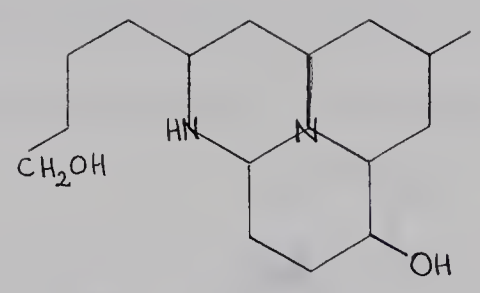


6c, M(264)

Compounds such as 9b, Table IX show a very rich fragmentation scheme since many different processes compete in the formation of reasonably stable ions. The spectrum of 9b shows fairly intense peaks at,

Table IX

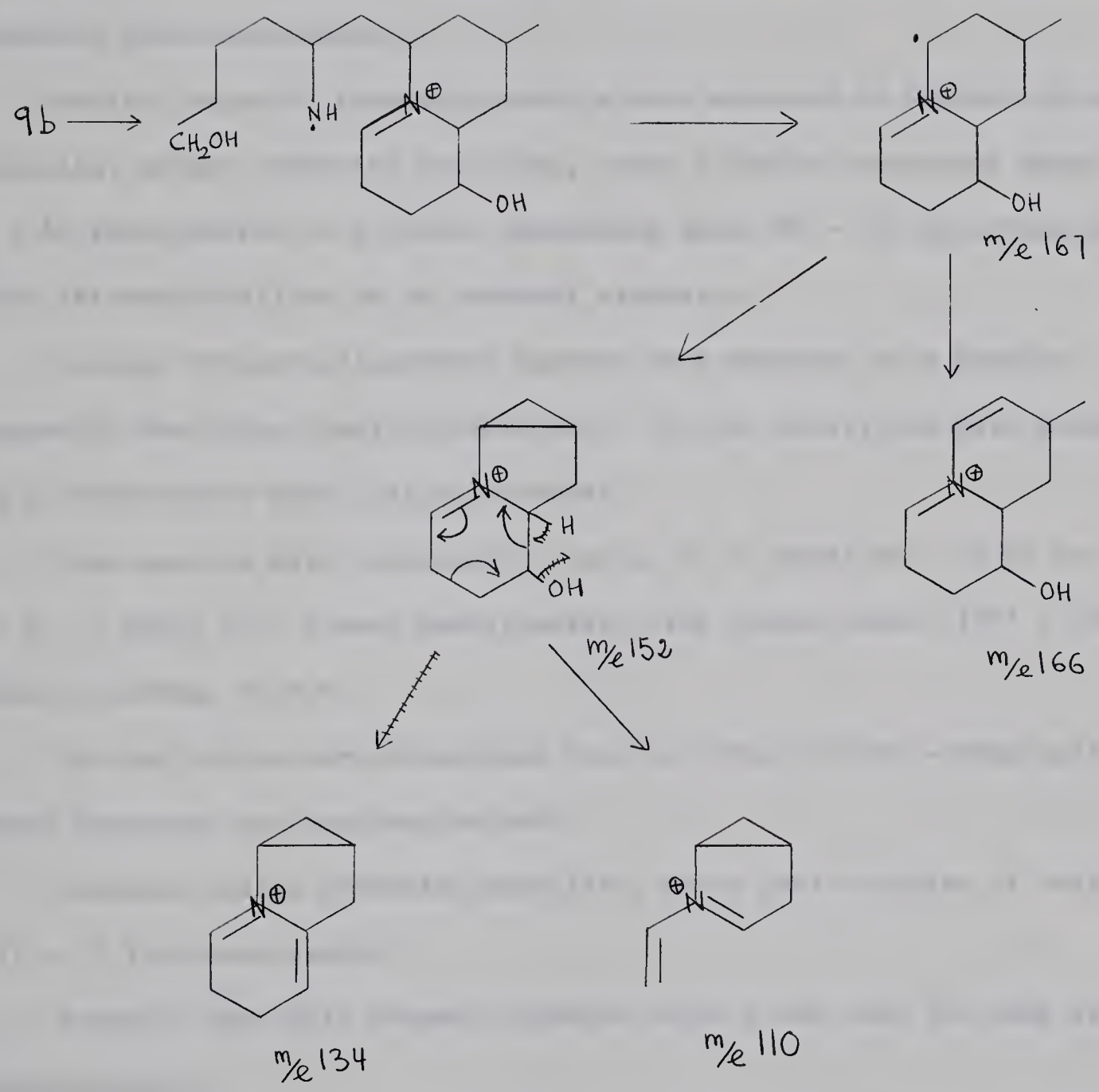
m/e	% B.P.	m/e	% B.P.	m/e	% B.P.
282	8	223	45	138	20
281	20	205	25	134	20
265	10	167	32	122	15
263	10	166	34	110	30
245	10	152	100	97	50
224	20	142	15	82	30



9b, M(282)

$M^{\oplus}$, m/e 282 (8); $M^{\oplus}$ - 1, m/e 281 (20); $M^{\oplus}$ - 17, m/e 265 (10); $M^{\oplus}$ - 18, m/e 264 (5); $M^{\oplus}$ - 19, m/e 263 (10); $M^{\oplus}$ - 59, 223 (45); $M^{\oplus}$ - 77, m/e 205 (25); $M^{\oplus}$ - 148, m/e 134 (20); $M^{\oplus}$ - 172, m/e 110 (30); m/e 97 (50); m/e 82 (30).

Most of these peaks can be explained in terms of the mechanisms explained earlier or are arrived to by straightforward derivation (e. g., $M^{\oplus}$ - 1; $M^{\oplus}$ - 17, $M^{\oplus}$ - 18; $M^{\oplus}$ - 19, $M^{\oplus}$ - 59; $M^{\oplus}$ - 77 (59 + 18), etc.); among the possible fragmentations to explain some of the remaining peaks are the following,


9b
CH2OH
NH
OH
m/e 167
H
OH
m/e 152
m/e 166
m/e 134
m/e 110

Experimental

Infrared spectra were recorded on a Perkin-Elmer Model 421 dual grating infrared spectrophotometer or a Perkin-Elmer Model 337 grating infrared spectrophotometer.

Nuclear magnetic resonance spectra were measured in deutero-chloroform solution, unless otherwise specified, using a Varian Associates Model A - 60 spectrometer or a Varian Associates Model HR - 100 spectrometer with tetramethylsilane as an internal standard.

Optical rotatory dispersion spectra were measured on a Rudolph Automatic Recording Spectropolarimeter. Optical activities were measured on a Perkin-Elmer Model 141 Polarimeter.

Mass spectra were determined on an A. E. I. Model MS - 2H or an A. E. I. Model MS - 9 mass spectrometer, with heated inlet (170° - 200°); electron energy 70 e.v.

Melting points were determined on a hot-stage Fischer-Johns melting point apparatus and are uncorrected.

Alumina, unless otherwise specified, means basic alumina of activity III - IV (Brockman scale).

Research Specialty Company aluminum oxide G was used for thin layer chromatography.

Microanalysis are by F. Pascher, Bonn, Germany; or by C. Daessle, Montreal, Quebec.

ISOLATION OF THE ALKALOIDS

Finely ground L. cernuum (11 kg. collected in Venezuela) was stirred at room temperature with methanol (ca. 10 ℓ) for 24 hours, filtered and the methanolic extract concentrated at reduced pressure. This process was repeated twice and the combined extracts were warmed with 3% aq. tartaric acid (ca. 3 ℓ). The tartaric acid insoluble portion was removed by filtration and then extracted again with tartaric acid (ca. 2 ℓ). The combined filtrates were extracted with ether then made strongly basic with ammonium hydroxide and extracted with chloroform. Evaporation of the chloroform and crystallization of the crude bases (12.0 g) from acetone, furnished lycocernuine (L-33)(2.07 g) which, after crystallization from acetone, melted at 230 - 31°, $[\alpha]_D$ - 24.5° (c 0.1, methanol). Calcd. for $C_{16}H_{26}O_2N_2$: C, 69.03; H, 9.41; N, 10.06%. Found: C, 69.09; H, 9.32; N, 9.86%. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$, 3615, 1620, 1410 cm^{-1} (see Fig. 1). Nuclear magnetic resonance spectrum (60 Mcs): τ 4.54 (1H, poorly resolved quartet, large splitting about 11 cps), 6.2 (1H, broad singlet), 9.13 (3H, doublet, splitting 6 cps).

Mass spectrum: m/e 278 (19, molecular ion), 277 (5), 250 (8), 249 (23), 233 (10), 221 (16), 220 (58), 219 (100), 205 (8), 191 (5), 166 (20), 165 (37), 152 (29), 151 (6), 138 (8), 124 (7), 122 (6), 110 (15), 108 (5).

The methiodide was prepared in methanol and recrystallized from acetone containing a few drops of methanol, m.p. 270 - 272°. Calcd. for $C_{16}H_{26}O_2N_2 \cdot CH_3I \cdot \frac{1}{2}H_2O$; C, 47.55; H, 7.04%. Found: C, 47.59, 47.89; H, 7.25, 7.54%. The lycocernuine was identical (infrared spectrum, mixed m.p.) with an authentic sample of alkaloid L-33[74].

The mother liquors from the crystallization were dissolved in benzene and subjected to chromatography over basic alumina (200 g., activity III).

Elution with benzene, and benzene-ether (1:1), furnished 0.2 g. of a mixture of bases (see below). Elution with ether gave cernuine (1.1 g), and elution with chloroform gave first a mixture of cernuine and lycocernuine (0.3 g) and then lycocernuine (0.7 g). Elution with chloroform-methanol (50:1 to 5:1) furnished a mixture of bases (2.1 g) which as yet has not been resolved into pure components.

Cernuine crystallized with difficulty from Skellysolve B and was most conveniently purified by sublimation at 100°/0.05 mm, m.p. 103 - 104°, $[\alpha]_D$ -20.5 (C 0.1, methanol). Calc. for $C_{16}H_{26}ON_2$: C, 73.24; H, 9.99%. Found: C, 73.04. 73.19; H, 9.95, 9.88%. Infrared spectrum: $\gamma_{max.}^{CCl_4}$ 1640; 1415 cm^{-1}. Nuclear magnetic resonance spectrum: τ 4.53 (quartet, splittings 11 cps and 2.5 cps), 6.3 - 7.1 (3H, complex series of peaks), 7.55 - 7.80 (2H, multiplet), 9.14 (3H, doublet, splitting 6 cps). Mass spectrum (only the 14 most intense peaks listed): m/e 262 (49, molecular ion), 261 (15), 247 (15), 234 (26): 233 (100), 221 (16), 220 (91), 219 (57), 205 (28), 191 (24), 178 (15), 164 (20, 151 (21), 150 (20).

Further supplies of crude alkaloids from plant material collected in Mexico were obtained from Smith, Kline and French Laboratories, Philadelphia, and gave similar proportions of cernuine and lycocernuine.

DIHYDRODEOXYLYCOCERNUINE (12a)

Lycocernuine (40 mg.) was added to a suspension of lithium aluminum hydride (40 mg) in anhydrous tetrahydrofuran (20 ml). [Anhydrous tetrahydrofuran refers to freshly distilled T. H. F., which has been first

heated under reflux in the presence of excess lithium aluminum hydride]. The resulting mixture was heated under reflux for 20 hr. then worked up using the method of Mićović and Mahailović[81].

Dihydrodeoxylycocernuine, after recrystallization from acetone, melted at 193 - 194°. The compound was analyzed by high resolution mass spectrometry. Calc. for $C_{16}H_{28}ON_2$; molecular weight 264.2202. Found: 264.2207.

Infrared spectrum: $\gamma_{max.}^{Nujol}$ 3160 cm^{-1} (broad). Bohlmann bands (see Fig. 3).

Nuclear magnetic resonance spectrum: τ 6.20 (1H, poorly resolved multiplet), 6.40 (1H, quartet, J = 11.5 and 2.5 cps), 9.17 (3H, doublet, splitting 6.5 cps).

DEUTERIUM EXCHANGE WITH LYCOCERNUINE

Lycocernuine (20 mg) was added to a solution of sodium (40 mg) in methanol-O-d (2 ml). The resulting solution was left at room temperature for 24 hours and then was refluxed for 8 hours. The solvent was evaporated and the residue distributed between deuterium oxide and chloroform. Evaporation of the chloroform and crystallization of the residue from wet acetone yielded deuterated lycocernuine (2h) (see p. 76), the mass spectrum of which indicated the presence of 75% dideuterated and 21% monodeuterated lycocernuine. Infrared spectrum: $\gamma_{max.}^{Nujol}$ 3200, 1635 cm^{-1}. The band appearing at 1410 cm^{-1} in the spectrum of lycocernuine is not present in the spectrum of the deuterated compound.

O-ACETYLLYCOCERNUINE (21)

Lycocernuine (28 mg) was dissolved in pyridine-acetic anhydride (3 ml, 1:1), and left overnight at room temperature. The solvents were removed *in vacuo* and the residue filtered through a short column of alumina. O-Acetyllycocernuine could not be induced to crystallize and was purified by molecular distillation at 180°/0.1 mm. Calcd. for $C_{18}H_{28}O_3N_2$: molecular weight, 320. Found: 320 (mass spec.). Infrared spectrum: $\gamma_{max.}^{CCl_4}$ 1730, 1640, 1410, 1230 cm^{-1}. Nuclear magnetic resonance spectrum: τ 4.45 (1H, quartet, splitting 11.5 and 3 cps), 5.06 (1H, poorly resolved triplet), 7.91 (3H, singlet), 9.14 (3H, doublet, splitting 6.0 cps)(see Fig. 2).

DEHYDROLYCOCERNUINE (7)

A. Jones' method[39]. A solution of lycocernuine (84 mg) in acetone (25 ml) was cooled in an ice bath and Jones' reagent* (0.08 ml) added. After twenty minutes most of the solvent was removed at the pump, water (5 ml) was added and the resulting solution extracted with chloroform (6 x 5 ml.). Evaporation of the chloroform yielded dehydrolycocernuine (48 mg). Basification of the aqueous solution and extraction with chloroform yielded 30 mg. of a mixture of lycocernuine and dehydrolycocernuine (t.l.c. analysis) which on further oxidation furnished 20 mg. of the ketone. Dehydrolycocernuine, purified by sublimation at 120°/0.1 mm, melted at 161 - 164°. Calcd. for $C_{16}H_{24}O_2N_2$: C, 69.53; H, 8.75%; molecular wt.,

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* Convenient preparations of the reagent are described by Bible[82] and Johnson[83] et al.

276. Found: C, 69.74; H, 8.87; molecular weight 276 (mass spec.). Infrared spectrum: $\gamma_{max.}^{Nujol}$ 1705, 1635, 1410 cm^{-1}.

Nuclear magnetic resonance spectrum: τ 3.91 (1H, poorly resolved quartet), 6.28 (1H, poorly resolved triplet), 9.10 (3H, doublet, splitting 6 cps).

Rotatory dispersion in methanol (c 0.23); $[\alpha]_{589}$ + 10 ± 5°, $[\alpha]_{400}$ + 45°, $[\alpha]_{339}$ + 3140°, $[\alpha]_{292}$ - 3840°, $[\alpha]_{275}$ - 3600°.

B. Oxidation with chromic acid-acetic acid.

A suspension of chromium trioxide (100 mg) in glacial acetic acid (10 ml) was cooled in an ice bath and lycocernuine (35 mg) added. The solution was stirred with a magnetic stirrer and taken occasionally out of the ice bath to avoid solidification. After one hour the contents of the flask were poured dropwise into dilute aqueous ammonium hydroxide. The resulting solution was extracted with chloroform. Evaporation of the chloroform afforded a gummy residue. This residue was purified by chromatography on alumina. Elution with ether afforded dehydrolycocernuine (9 mg).

C. Dimethyl sulfoxide-dicyclohexylcarbodiimide method

Lycocernuine (30 mg, 0.1 millimoles) and dicyclohexyl carbodiimide (62 mg, 0.3 millimoles) were dissolved in dimethyl sulfoxide (6 ml) and phosphoric acid (23 mg, 0.2 millimoles) added. The solution was left overnight at room temperature.

The dicyclohexylurea formed was filtered off. The dimethyl sulfoxide solution was diluted with water (20 ml) and extracted with chloroform (4 x 10 ml). This chloroform extract yielded no product. The aqueous solution was made basic with aqueous sodium carbonate and extracted again

with chloroform. Evaporation of the chloroform extract furnished lycocernuine (21 mg).

D. Oxidation with pyridine-chromium trioxide

A solution of lycocernuine (20 mg) in pyridine (3 ml) was added to a flask containing chromium trioxide (100 mg) and pyridine (3 ml). The flask was kept 2 hours at 0° C and for another 5 hours at room temperature.

Ethanol (1 ml) was added to destroy the pyridine-chromium trioxide complex. The solution was further diluted with water (20 ml). The aqueous solution was extracted with ether (4 x 10 ml). The ethereal extract (strongly colored) was washed with aqueous sodium bicarbonate and distilled water. Evaporation of the ether did not afford any basic or neutral material.

The aqueous solution was basified with aqueous sodium carbonate and extracted with chloroform (2 x 10 ml). No residue was obtained from the chloroform extract.

SODIUM BOROHYDRIDE REDUCTION OF DEHYDROLYCOCERNUINE

Sodium borohydride (45 mg) and sodium carbonate (100 mg) were added to a solution of dehydrolycocernuine (45 mg) in ethanol (10 ml) and the resulting mixture was stirred overnight at room temperature, then most of the solvent was removed at the pump and water was added.

Extraction with $CHCl_3$ yielded a mixture (45 mg) of two products (t.l.c.). Crystallization of the mixture from acetone-ether furnished lycocernuine (2b) (20 mg), m.p. 212 - 213°, identical (infrared spectrum in chloroform) with an authentic sample.

Chromatography of the material obtained from the mother liquors over

alumina (2 g, activity III) gave more lycocernuine (10 mg) eluted with ether-chloroform (9:1) and then epilycocernuine. The mass spectrum of epilycocernuine shows a molecular ion at m/e 278 and is very similar to that of lycocernuine. The infrared spectrum (Nujol mull) shows hydroxyl absorption at 3460 cm^{-1} and lactam carbonyl absorption at 1635 cm^{-1}.

WOLFF-KISHNER REDUCTION OF DEHYDROLYCOCERNUINE

Since dehydrolycocernuine is sensitive to air especially in alkaline solution, it is essential to conduct this reaction in an inert atmosphere.

Hydrazine hydrate (0.5 ml) was added to a solution of dehydrolycocernuine (50 mg) in dethylene glycol (5 ml) in a nitrogen atmosphere. The solution was heated at 100 - 130° for one hour, a pellet of sodium hydroxide was then added and the temperature raised to 180 - 190° for three hours. The cooled solution was diluted with water and extracted several times with chloroform. The chloroform solution was washed with water, evaporated at the pump, and the residue, which contained traces of diethylene glycol was taken up in chloroform and filtered through alumina. Evaporation of the chloroform yielded cernuine (2a) (30 mg), m.p. (after sublimation) 103 - 104° identical (infrared spectrum, mixed m.p., t.l.c. behaviour) with an authentic sample.

Various other modifications of the Wolff-Kishner reduction method were used in several attempts to correlate lycocernuine and cernuine. Some of them will be described below.

A. The method employed is that described by Cram[45]. Dehydrolycocernuine (55 mg) was added to a solution of absolute hydrazine (two drops) in absolute ethanol. The ethanolic solution was heated under reflux for three hours. Evaporation of the solvent afforded the dehydrolycocernuine

hydrazone. Infrared spectrum ($CHCl_3$): 3425 and 1650 cm^{-1}, t.l.c. analysis only one component.

The residue (50 mg) obtained above was dissolved in anhydrous dimethyl sulfoxide (50 ml) and this solution was slowly added to a vigorously stirred solution of sublimed potassium tert-butoxide (60 mg) in anhydrous dimethyl sulfoxide (10 ml). The mixture was stirred at room temperature for twelve hours. The solution was reduced to small volume in vacuo, diluted with water and extracted with chloroform. Evaporation of the chloroform afforded only dehydrolycocernuine hydrazone (t.l.c.) (40 mg).

B. Henbest's method[44]. The dehydrolycocernuine hydrazone was prepared as described in section A.

Dehydrolycocernuine hydrazone (50 mg) was dissolved in toluene (10 ml) and sublimed potassium tert-butoxide (60 mg) added. The mixture was heated under reflux for fifteen hours. The residues obtained from aliquots removed from the reaction mixture at five hour intervals showed (t.l.c.) that the amount of cernuine formed during the first five hours did not appreciably increase thereafter. The solvent was removed in vacuo; the residue was dissolved in water and extracted with chloroform. The complex mixture obtained by evaporation of the chloroform was subjected to chromatography on alumina. Elution with ether-chloroform (1:1) afforded cernuine (4 mg). Cernuine, obtained in this manner, was purified by sublimation (110°, 0.1 mm Hg) but failed to crystallize. The infrared spectrum was very similar to one of authentic material.

C. Sodium borohydride reduction of the dehydrolycocernuine p-toluenesulfonyl hydrazone.

The method employed is that described by Cagliotti and Grasselli[46].

The p-toluensulfonyl hydrazone was prepared adding dehydrolycocernuine (40 mg, 0.14 mmoles) to a solution of p-toluensulfonyl hydrazine (40 mg, 0.2 mmoles) in methanol and heating the mixture under reflux for 4 hours. Evaporation of most of the solvent in vacuo left a gummy residue. Recrystallization of this residue from ether containing a few drops of methanol afforded the solid hydrazone. M.P. 150°, dec. The product was a single component of polarity greater than dehydrolycocernuine (t.l.c.).

The crude p-toluensulfonyl hydrazone, obtained above, was added to a solution of sodium borohydride (80 mg) in methanol (20 ml). This mixture was heated under reflux for 8 hours. Most of the solvent was evaporated and the residue dissolved in water and extracted with chloroform. Evaporation of the chloroform afforded a mixture of four basic components (t.l.c.) (20 mg), one of them of same R_F value as cernuine. The mixture was chromatographed on basic alumina. Elution with chloroform furnished cernuine (10 mg) contaminated with a second component. This mixture (10 mg) was separated by preparative t.l.c. on an alumina plate (0.25 mm thickness developed with chloroform). Cernuine (4 mg) was isolated. After further purification by sublimation (110°, 0.1 mm Hg) an infrared spectrum was obtained that proved to be practically identical to that of cernuine. However, the product failed to crystallize, and did not give a crystalline methiodide.

DEUTERIUM LABELLING

A. Deuterium exchange with dehydrolycocernuine (7)

Dehydrolycocernuine (15 mg) was dissolved in a solution of deuterium chloride in acetic acid-O-d (stock solution prepared by adding a 38%

solution of deuterium chloride in deuterium oxide (10 g) dropwise to cold (0°) acetic anhydride (31.6 g)) and kept at room temperature for ten hours, after which time the solvent was evaporated.

Since analysis of deuterium content of the ketone was complicated by exchange in the inlet system, the crude product was dissolved in methanol-O-d, sodium carbonate (25 mg) and sodium borohydride added and the mixture left overnight at room temperature. The product was isolated in the usual manner and crystallized from acetone to yield deuterated lycocernuine, m.p. 230 - 231°.

Mass spectrometry indicated the presence of 74% trideuterated and 21% dideuterated species, accompanied by small amounts of mono and tetra-deuterated material.

ANHYDROLYCOCERNUINE (4)

Lycocernuine (40 mg) in pyridine (3 ml) containing methanesulfonyl chloride (0.5 ml) was kept at 5° for three days. The cold solution was then diluted with dilute sodium hydroxide and extracted several times with ether. The crude product obtained by evaporation of the ether was heated under reflux with methanolic sodium methoxide (60 mg sodium in 3 ml methanol) for three hours, then the solution was concentrated at the pump, water added, and the resulting solution extracted several times with ether.

The product, anhydrolycocernuine (26 mg), showed a single spot on t.l.c. and after sublimation (120°, 0.1 mm) melted at 140 - 142°. Calculated for $C_{16}H_{24}ON_2$: C, 73.91; H, 9.30; N, 10.78%. Found: C, 73.42; H, 8.83; N, 11.51. Molecular wt., 260 (mass spectrometry). Infrared spectrum: $\gamma_{max.}^{Nujol}$, no OH absorption, 1655, 1635 cm^{-1}. Nuclear

magnetic resonance spectrum: τ 4.41 (1H, quartet, splittings 11 and 2 cps), 5.27 (1H, broad singlet), 6.49 (1H, multiplet), 9.03 (3H, doublet, splitting 6.3 cps).

ATTEMPTED TOSYLATION OF LYCOCERNUINE

Lycocernuine (28 mg, 0.1 mmoles) was dissolved in pyridine (3 ml) and p-toluensulfonyl chloride (27 mg, 0.15 mmoles) added. This mixture was kept at 5° for forty eight hours. The solvent was removed at the pump, the residue dissolved in water, cooled and then basified with dilute ammonia. The aqueous basic solution was extracted with ether (3 x 10 ml). The residue obtained from the ether extract (8 mg) was constituted of two components. One of the components had the same R_F as anhydrolycocernuine.

Further extraction of the aqueous solution with chloroform yielded lycocernuine (15 mg).

The two component mixture was separated by preparative t.l.c. on alumina plate (0.5 mm thickness). The product, anhydrolycocernuine (2 mg) showed a molecular weight of 260 and a fragmentation pattern corresponding to anhydrolycocernuine. No tosyl lycocernuine was isolated.

Carrying out the reaction at 115° for four hours did not alter this result.

The dehydration of lycocernuine was attempted several times before a successful method was found. Some of these attempts are described below,

A. Lycocernuine (40 mg) was dissolved in pyridine (2 ml) and phenylphosphonyl dichloride (1 ml) added to the solution. The mixture was heated (60°) for sixteen hours with stirring. Without cooling, the excess phenylphosphonyl dichloride was destroyed with addition of water (10 ml).

During this process a black solid separated from the solution. This compound, insoluble in all the usual organic solvents could not be characterized. Extraction with chloroform of the aqueous solution did not afford any basic material.

B. Lycocernuine (25 mg) was transferred to a 10 cm x 8 mm glass tube sealed in one end. Pyridine-modified alumina (100 mg) [this alumina was prepared with alumina (Woelm, activity I)(2g) and pyridine (0.04 ml), shaking this mixture in a wrist-action mechanical shaker for five hours] was added to the tube. Both solids were well mixed and then the lower end of the tube was immersed in a oil bath at 240°. After one hour the tube was cooled and the solid digested first with ether and finally with chloroform. The ethereal solution did not afford any basic product. From the chloroform, lycocernuine (11 mg) was the only product obtained.

ATTEMPTED PYROLYSIS OF O-ACETYLLYCOCERNUINE

O-Acetyllycocernuine (30 mg) was introduced into a glass tube sealed at one end. The tube was then filled with glass beads to a length of thirteen centimeters. The tube was inserted into a metallic block at 300°. The product that distilled over (10 mg) proved to be identical with starting material (infrared spectrum, t.l.c.).

ALLOCERNUINE (5)

Anhydrolycocernuine (20 mg) in ethyl acetate (5 ml) was hydrogenated at atmospheric pressure in the presence of Adams' catalyst (10 mg). After five hours the catalyst was removed and the solvent evaporated. The product showed two spots on t.l.c., a minor one corresponding in

R_F to cernuine, and a major component of higher R_F value. The minor component was removed by chromatography over alumina and the major component, allocernuine, which was obtained as a colorless oil which could not be induced to crystallize, was purified by molecular distillation at 120°/0.5 mm. Calcd. for $C_{16}H_{26}ON_2$: molecular wt., 262.2045. Found: 262.2041. Infrared spectrum: (see Fig. 4) $\gamma_{max.}^{CCl_4}$ 1625, 1415 cm^{-1}, fingerprint region distinctly different from that of cernuine.

Nuclear magnetic resonance spectrum: τ 5.01 (1H, quartet, splittings 10 and 2.5 cps), 9.05 (3H, doublet, splitting 5.5 cps). Mass spectrum (only the fifteen most intense peaks listed): m/e 262 (26), 261 (15), 247 (7), 234 (18), 233 (100), 220 (15), 219 (33), 205 (10), 192 (7), 191 (11), 165 (9), 164 (13), 151 (14), 150 (20), 136 (9).

DIHYDROALLOCERNUINE (6a)

A. From anhydrolycocernuine (4).

A solution of anhydrolycocernuine (70 mg) in methanol (6 ml) was hydrogenated at atmospheric pressure for twenty-four hours in the presence of 5% palladium-charcoal (70 mg). The catalyst was filtered off, washed several times with warm methanol, and the methanol removed at the pump. The product (65 mg), which showed three spots on t.l.c., was chromatographed over alumina (2.5 g, activity III). Elution with benzene-ether (1:1) provided allocernuine (13 mg), elution with ether gave trace amounts of a compound whose R_F value was identical with that of cernuine, and elution with chloroform yielded dihydroallocernuine (40 mg), m.p. 99 - 100°. Calculated for $C_{16}H_{28}ON_2$: molecular wt., 264. Found: molecular wt., 264

(mass spect.). Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 3400, 1645 cm^{-1}. Nuclear magnetic resonance spectrum: τ 3.70 (1H, broad, amide NH), 6.3 - 6.7 (3 - 4H, complex multiplet), 9.07 (3H, doublet, splitting 6 cps). Mass spectrum: m/e 264 (3), 166 (4), 153 (19), 152 (100), 110 (100).

B. From allocernuine (5).

Allocernuine (16 mg) was added to a solution of $NaBH_4$ (20 mg) in methanol (5 ml) containing potassium carbonate (20 mg). The mixture was left overnight, then since t.l.c. analysis of an aliquot indicated the presence of some starting material, more $NaBH_4$ (20 mg) was added and the mixture was refluxed for 2 hours. Work up in the usual manner yielded a mixture (10 mg) of starting material (identified by t.l.c., minor component) and dihydroallocernuine (major component). The dihydroallocernuine (6 mg) was isolated in pure form by chromatography of the mixture over alumina and was shown to be identical with that prepared by method A.

Catalytic reduction of allocernuine in methanol also furnished dihydroallocernuine.

$LiAlH_4$ REDUCTION OF DIHYDROALLOCERNUINE (6a)

Dihydroallocernuine (30 mg) was allowed to react with $LiAlH_4$ (40 mg) in refluxing ether for 10 hours, then worked up in the usual manner. The crude product (23 mg) was chromatographed over alumina. Elution with chloroform yielded 6b as an oil (17 mg) which showed a single spot on t.l.c. The analytical sample of 6b was prepared by molecular distillation at 120°/0.1 mm. Calcd. for $C_{16}H_{30}N_2$: molecular wt. 250.2409. Found: molecular wt., 250.2411. The infrared spectrum showed very weak NH absorption at 3300 cm^{-1} and no carbonyl absorption. Mass spectrum:

m/e 250 (5), 205 (8), 166 (9), 153 (11), 152 (100), 124 (11), 110 (18), 97 (16), 84 (25).

PREPARATION OF COMPOUND 6c

Compound 6b (above, 30 mg) was refluxed with formic acid - formaldehyde (1 ml of 1:1) for twelve hours. The solution was then diluted with water, made basic with dilute sodium hydroxide, and extracted several times with ether. The product was an oil which showed a single spot on t.l.c. (R_F value greater than that of 6b) and was further purified by molecular distillation at 120°/0.1 mm.

Calcd. for $C_{17}H_{32}N_2$: molecular wt., 264 Found: molecular wt., 264 (mass spect.). The infrared spectrum showed no NH or carbonyl absorption but showed an $N-CH_3$ band at 2790 cm^{-1}. Nuclear magnetic resonance spectrum: τ 7.70 (3H, singlet), 9.10 (3H, doublet, splitting 4.5 cps). Mass spectrum: m/e 264 (9), 249 (5), 178 (8), 166 (10), 153 (11), 152 (100), 124 (6), 112 (6), 111 (11), 110 (8), 98 (50).

DIHYDRODEOXYEPIALLOCERNUINE (17)

A. From epiallocernuine (see following experiment)

B. From allocernuine

Lithium aluminum hydride (40 mg) was added to an ethereal solution of allocernuine (40 mg) and the mixture heated under reflux for ten hours. Excess lithium aluminum hydride was destroyed in the usual manner, the granular precipitate was filtered off and the filtrate washed with water, dried and evaporated. The residue (17) was a crystalline compound, m.p. 96 - 97° (t.l.c. analysis showed a single component). Yield 32 mg.

Calculated for $C_{16}H_{28}N_2$, molecular weight 248.2253. Found: 248.2249. Infrared spectrum (see Fig. 6). No OH or NH absorption. No carbonyl absorption. Bohlmann bands.

Other fragments in the mass spectrum: 247 (35), 220 (22), 219 (100), 206 (25), 205 (30), 164 (5), 150 (10), 84 (9), 55 (10).

EPIALLOCERNUINE (15)

A solution of allocernuine (110 mg) in methanol (10 ml) was heated under reflux for 72 hours. Evaporation of the methanol *in vacuo* left a residue made up of two components, allocernuine and epiallocernuine (t.l.c.).

The mixture of allo- and epiallocernuine was chromatographed on alumina (3 g). Elution with benzene afforded allocernuine (15 mg). Dilution with ether yielded a mixture of allo- and epiallocernuine (20 mg) and further elution with ether gave pure epiallocernuine (65 mg). Epiallocernuine solidified when the solvent was thoroughly pumped off. M.P. 74 - 75° C.

Calculated for $C_{16}H_{26}NO_2$: 262.2045. Found: 262.2041. The mass spectrum was similar to those of cernuine and allocernuine. Infrared spectrum: $\gamma_{max.}^{CCl_4}$ No OH or NH absorptions, 1665, 1420 cm^{-1}. Strong Bohlman's bands (see Fig. 5). Nuclear magnetic resonance spectrum: τ 9.2 (3H, doublet, splitting 6 cps). No signal below τ 6.0.

DIHYDRODEOXYEPIALLOCERNUINE

Epiallocernuine (6 mg) was added to a slurry of lithium aluminum hydride (10 mg) in ether. The mixture was heated under reflux overnight.

Working up the reaction in the usual manner gave dihydrodeoxyepiallocernuine (17) (4.5 mg) as a crystalline solid. The identity was proved by its infrared spectrum and t.l.c. behaviour.

MERCURIC ACETATE OXIDATION OF LYCOCERNUINE:

LYCOCERNUINE OXAZOLIDINE (10)

Mercuric acetate (200 mg) was placed in a flask containing aqueous acetic acid (5%, 20 ml.) and lycocernuine (75 mg) added. The flask was heated on the steam bath for 10 hours. An inert atmosphere was maintained by bubbling nitrogen through the solution during the complete reaction period. The mercurous acetate precipitate was filtered off. The aqueous solution was saturated with hydrogen sulfide and the black precipitate of mercuric sulfide eliminated by filtration. The aqueous filtrate was basified with aqueous ammonium hydroxide and extracted with chloroform.

Evaporation of the chloroform left a residue constituted by a single product (t.l.c.). The lycocernuine oxazolidine was unstable to air and failed to crystallize. It also decomposed on attempted molecular distillation. Purification was achieved by passing it through a short alumina column. Exposure to air, even for short periods of time caused rapid discoloration of this compound. Calcd. for $C_{16}H_{24}N_2O_2$: 276.1838. Found for $C_{16}H_{24}N_2O_2$: 276.1841. Infrared spectrum (CCl_4): no OH absorption, 1640 cm^{-1}. Nuclear magnetic resonance spectrum: τ = 4.4 (1H, poorly resolved multiplet), 5.8 (1H, doublet, splitting 4 cps), 6.0 (1H, poorly resolved multiplet), 6.88 (1H, triplet, splitting 3 cps), 9.1 (3H, doublet, splitting 6 cps).

ACETYL DERIVATIVE OF 10

The oxazolidine (10) (55 mg) was dissolved in a mixture of pyridine and acetic anhydride (1:1, 6 ml). This solution was left overnight at room temperature. Most of the solvent was evaporated at the pump, the residue dissolved in water and the aqueous solution extracted with chloroform. Compound 11 (see p. 55) was the only product of this reaction (t.l.c.). The crude product was further purified by passing it through a short alumina column. The purified product was a yellowish oil that failed to crystallize. Calcd. for $C_{20}H_{28}O_4N_2$: molecular wt., 360. Found: molecular wt., 360 (mass spect.). Ultraviolet spectrum: $\gamma_{max.}$ (EtOH) 322mμ, log.ε 3.43 Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 1720, 1680, 1640 cm^{-1}. No OH absorption. Nuclear magnetic resonance spectrum: τ 4.3 (1H, quartet, splittings 10 and 2 cps). 5.1 (1H, width at half-height 6 cps), 5.4 (1H, quartet, splittings 10 and 4 cps), 6.4 (1H, complex multiplet), 6.85 (1H, doublet, splitting 13 cps), 7.76 (3H, singlet), 8.0 (3H, singlet), 9.05 (3H, doublet, splitting 6 cps).

HYDROGENATION OF 11

A solution of 11 (50 mg) in methanol (6 ml) was hydrogenated for ten hours at atmospheric pressure in the presence of palladium charcoal (50 mg). The catalyst was removed and the solvent evaporated. The crude product obtained was made up of two components. These components, probably epimeric dihydroderivatives of 11, failed to separate when chromatographed over alumina. No separation could be obtained by molecular distillation (120°/0.1 mm) of the mixture. Calculated for $C_{20}H_{30}O_4N_2$: molecular wt., 362. Found: molecular wt., 362 (mass spec.). Ultraviolet spectrum:

No characteristic absorption. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 1720, 1700 and 1640 cm^{-1}. N.m.r. spectrum: τ 4.8 (1H, poorly resolved multiplet) 5.15 (1H, poorly resolved multiplet) 7.6 (3H, singlet), 7.85 (3H, singlet), 9.02 (3H, doublet, splitting 7 cps). Mass spectrum: 362 (9), 320 (18), 319 (80), 306 (12), 305 (65), 291 (40), 262 (22), 261 (100), 259 (17), 219 (21), 166 (17), 150 (14), 148 (20), 146 (20), 133 (23), 98 (25).

PERIODATE-PERMANGANATE OXIDATION OF ANHYDROLYCOCERNUINE

The oxidation was carried out following the Lemieux-von Rudloff method[55]. A stock solution was prepared by dissolving sodium periodate (2.086 g) and potassium permanganate (39.5 mg) in distilled water (100 ml).

A mixture of the stock solution (20 ml), distilled water (50 ml) and pyridine (25 ml), in which a small amount of potassium carbonate (25 mg) had been dissolved, was added slowly to a solution of the olefin 13 (60 mg) in pyridine. The permanganate color dissappeared quite rapidly, the color of the solution changing to a red-wine tone. After five minutes the oxidation was interrupted by adding an aqueous solution of sodium metabisulfite to the reaction mixture. The aqueous solution turned colorless; it was left standing for one hour and evaporated to half volume *in vacuo*.

The aqueous solution was made acidic with sulfuric acid (10%) and was continuously extracted from ether. The acidic product obtained by evaporation of the ethereal extracts was transformed into its methyl ester. This ester 19 (15 mg), was identical (t.l.c., mass-spectrum and n.m.r.) to the product obtained by aerial oxidation of dehydrolycocernuine (see below).

AERIAL OXIDATION OF DEHYDROLYCOCERNUINE

Dehydrolycocernuine (50 mg) was suspended in 10% aqueous sodium hydroxide (10 ml) and enough methanol was added to dissolve the ketone. A stream of air was bubbled through the stirred solution for twenty hours, at which time it was diluted with water and extracted with chloroform to remove unreacted starting material. The aqueous layer was then acidified with sulfuric acid and extracted with chloroform to give the crude acid 20 as a solid (m.p. 72 - 75°) which showed a single spot on t.l.c. (silica gel plate). Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 3500, 2600, 1720, 1650 and 1600 cm^{-1}.

Without further characterization this acid was converted into its methyl ester by treatment with diazomethane. The ester crystallized from ether, m.p. 103 - 105°. Calcd. for $C_{17}H_{26}O_4N_2$: molecular weight, 322. Found: molecular weight, 322. Infrared spectrum: $\gamma_{max.}^{Nujol}$ 1725, 1635, 1410 cm^{-1}. Nuclear magnetic resonance spectrum: τ 2.82 (1H, triplet, splitting 7 cps), 6.35 (3H, singlet), 7.2 (2H, doublet, splitting 5 cps), 9.02 (3H, doublet, splitting 5.5 cps).

OZONOLYSIS OF ANHYDROLYCOCERNUINE (4)

A stream of oxygen containing ozone (generated in a Welsbach T - 23 apparatus) was bubbled for two hours through a solution of anhydrolycocernuine (70 mg) in ethyl acetate cooled in a dry ice-acetone bath.

The ethyl acetate solution was allowed to reach room temperature and then was poured into a flask containing hydrogen peroxide (30%, 1 ml) and sodium hydroxide (10%, 3 ml). This solution was heated for half an hour under reflux, concentrated *in vacuo* and extracted with chloroform to remove unreacted starting material. After acidification with dilute

hydrochloric acid (10%) the solution was extracted with chloroform and an acidic product (10 mg) collected. From its t.l.c. behaviour and infrared spectrum the product seemed to be identical to acid 20, obtained by aerial oxidation of dehydrolycocernuine.

PREPARATION OF 1-KETOQUINOLIZIDINE

The method employed is described in the literature[59] and is essentially based on the original synthesis of Clemo and Ramage[85].

A suspension of sodium ethoxide (2.9 g) in dry toluene (8 cc) was transferred to a predried three necked flask equipped with mechanical stirrer and gas inlet (through which nitrogen was being passed). To this mixture diethyl piperidyl-1-γ-butyrate-2 carboxylate (5.4 g) was added slowly while stirring. The reaction is slightly exothermic and by the time the addition was finished most of the catalyst was dissolved. The mixture was heated on the water-bath for two hours, then water added (3 cc) and made acidic with sulfuric acid (10 ml, 20%). The acidified solution was heated three hours on the steam-bath; the toluene layer was separated and the aqueous layer extracted with ether (50 ml). The aqueous layer was basified (NaOH, 30%) and extracted with ether. Evaporation of the ether left a residue that was fractionally distilled using a short Vigreau column. 1-Ketoquinolizidine was collected at 65°/2mm, t.l.c. showed a single spot. Infrared spectrum: $\gamma_{max.}^{CCl_4}$ 1715 cm^{-1} Bohlmann bands. The product decomposes on standing.

Sodium borohydride reduction of 1-ketoquinolizidine furnished 1-hydroxyquinolizidine m.p. 68 - 9°* Calculated for $C_9H_{17}NO$, molecular

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* The literature m.p. for the equatorial isomer is 71 - 72°.

weight 155. Found 155 (mass spectrometry). Infrared spectrum: $\gamma_{max.}^{CCl_4}$ 3635 and 3100 - 3400 cm^{-1} (broad band). No carbonyl absorption. Bohlmann bands.

AERIAL OXIDATION OF 1-KETOQUINOLIZIDINE

1-Ketoquinolizidine (80 mg) was added to 10% aqueous sodium hydroxide and enough methanol to make the solution homogenous. A stream of air was bubbled through the solution for 12 hours. The aqueous solution was acidified and extracted with chloroform. Evaporation of the chloroform yielded 30 mg. of acidic material; t.l.c. (on silica gel) showed only one spot. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 1710 and 1620 cm^{-1}. The acid was esterified by treatment with an ethereal solution of diazomethane. The ester was purified by molecular distillation (120°/0.1 mm). Infrared spectrum of ester (S) (see p. 33): $\gamma_{max.}^{CHCl_3}$ 1730 and 1625 cm^{-1}. Calculated for $C_{10}H_{17}NO_3$, molecular weight 199. Found 199 (mass spec.). Other important fragments: 198 (18), 167 (43), 139 (29), 125 (89), 111 (100).

REDUCTION OF LYCOCERNUINE WITH DISSOLVING METALS

A solution of lycocernuine (30 mg) in methanol (5 ml) was added dropwise to a flask containing liquid ammonia (50 ml.). This solution was vigorously stirred while lithium metal (0.2 g) was added at intervals to the flask in the course of thirty minutes. The ammonia was allowed to evaporate (removing the dry ice-acetone condenser) and the residue was dissolved in water. The aqueous solution was extracted with chloroform.

The residue, a single product (t.l.c.), was assigned structure 9b (see p. 48). Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 3620 cm^{-1}; no absorption in the CO

region. Calcd. for $C_{16}H_{30}O_2N_2$: molecular wt., 282. Found: molecular wt., 282 (mass spectrometry).

ACETYL DERIVATIVE OF 9b

The crude alcohol 9b (25 mg) was dissolved in a mixture (1:1) of pyridine:acetic anhydride (4 ml) and the solution left standing at room temperature for thirty hours.

This solution was worked up in the usual manner. The acetyl derivative (9c) obtained was purified by molecular distillation at 170° and 0.1 mm of Hg.

Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 1720, 1620 cm^{-1}. No OH or NH absorption. Nuclear magnetic resonance spectrum: τ 5.1 (1H, multiplet, width at half-height 6 cps); 5.3 (1H, multiplet, width at half-height: 11 cps); 5.9 (2H, triplet, splitting 5.5 cps), 7.9 (6H, singlet); 7.93 (3H, singlet); 9.1 (3H, doublet, splitting 5 cps).

REDUCTION OF DEHYDROLYCOCERNUINE WITH DISSOLVING METALS

A solution of dehydrolycocernuine (60 mg) in methanol (8 ml) was added to a flask containing liquid ammonia. Metallic lithium (0.2 g.), cut in small portions, was added to this mixture. Reaction time 30 minutes.

The reaction was worked up as described earlier. Evaporation of the chloroform extract left a gummy residue consisting of two components (t.l.c.). One of the components (the minor one) had R_F value identical with that of 9b. All attempts to purify this mixture failed to produce a pure component. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 3600, 3200 cm^{-1} (broad). No carbonyl absorption.

ACETYL DERIVATIVE OF 8

The crude mixture obtained above (40 mg) was dissolved in a pyridine-acetic anhydride mixture (1:1) (6 ml). This solution was left for thirty hours. Most of the solvent was evaporated *in vacuo*, the residue dissolved in water and this aqueous solution (pH ≃ 3) was extracted with ether. Evaporation of the ether yielded a residue (30 mg) which was mainly one component (9a). The aqueous solution was further basified (aqueous sodium bicarbonate) and extracted with chloroform. Evaporation of the chloroform afforded 8 mg. of a mixture of two components (t.l.c.) (9a and 9c). Compound 9a, obtained from the ethereal extracts was purified by molecular distillation (170 - 180°, 0.1 mm). Mass spectrum: Highest mass ion at m/e: 366 ($M^{\oplus}$ - 42). Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 1720, 1620 cm^{-1}. No OH or NH absorption. Nuclear magnetic resonance spectrum: τ 4.8 (1 H, sextet, splitting 4 cps); 5.26 (1H, multiplet, width at half-height 13 cps); 5.9 (2H, triplet, splitting 5.5 cps); 7.9 (3H, singlet); 7.95 (6H, singlet); 9.1 (3H, doublet, splitting 5 cps).

REACTION OF LYCOCERNUINE WITH ETHYLBROMOACETATE

Lycocernuine (28 mg) was dissolved in hot benzene. To this solution ethyl bromoacetate (1 ml) was added. The mixture was refluxed for fourteen hours. The solvent was evaporated and the last traces of ethyl bromoacetate eliminated under high vacuum. The crystalline residue was washed with a small amount of cold acetone and used in an attempt to prepare the lactone salt of lycocernuine. Infrared spectrum: $\gamma_{max.}^{Nujol}$ 1725 and 1640 cm^{-1}, OH absorption.

ATTEMPTED LACTONIZATION OF THE ESTER SALT X

The ester salt obtained above was dissolved in aqueous hydrobromic acid (4N) (2 ml) and the solution was heated under reflux for eight hours. The solvent was eliminated *in vacuo* and the residue was recrystallized from acetone-methanol. No evidence could be gathered to support the formation of the lactone. The infrared spectrum showed only carboxyl and lactam carbonyl absorption and no absorption due to a lactone carbonyl could be detected.

TREATMENT OF LYCOCERNUINE WITH HYDRIODIC ACID[48]

Lycocernuine (20 mg) was dissolved in hydriodic acid (54 - 56%) (3 ml) (the hydriodic acid had been freshly distilled from red phosphorous). This solution was heated under reflux for ten hours. The solution was neutralized with concentrated ammonium hydroxide and extracted with ether. No residue was obtained by evaporation of the ether extract. The aqueous basic solution was further extracted with chloroform. By evaporation of the chloroform lycocernuine was obtained (m.p. and mixed m.p's).

An increase of the reaction time (sixty hours) led to poor recovery of lycocernuine as the only product. Milder conditions (room temperature for four days) failed to produce any compound other than lycocernuine.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF ESTER 19

The ester (30 mg) was dissolved in ether (15 ml), lithium aluminum hydride (80 mg) was added to the solution and this mixture was heated under reflux for twelve hours and then left overnight at room temperature.

By working up the reaction in the usual manner an oily residue (17 mg) was isolated. This residue consisted of a single product 21 of slightly lower R_F than the starting material. Calculated for $C_{16}H_{30}ON_2$, molecular weight 266. Found, molecular weight, 266 (mass spectrometry); base peak at m/e: 207. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 3620 cm^{-1}. No carbonyl absorption.

CLEMMENSEN REDUCTION OF DEHYDROLYCOCERNUINE

Dehydrolycocernuine (40 mg) was dissolved in aqueous hydrochloric acid (~ 8 N), amalgamated zinc[43] was added to this solution and the mixture was heated on the steam bath for twelve hours.

The solids were removed by filtration. The aqueous solution was basified (aqueous ammonia) and extracted with chloroform. The residue obtained by evaporation of the chloroform extract was extremely complex (t.l.c.). Attempts to isolate pure components by chromatography failed. The various fractions isolated exhibited OH and/or NH absorption in their infrared spectrum.

ATTEMPTED REDUCTION OF DEHYDROLYCOCERNUINE WITH ZINC-ACETIC ACID

The method used has been described in the literature[41]. The ketone (30 mg) was dissolved in acetic acid (5 ml) and zinc-dust (200 mg) was added to the solution. The mixture was stirred for two hours at room temperature. The solids were eliminated by filtration and the acetic acid neutralized using concentrated ammonium hydroxide. The solution was then extracted with chloroform. Evaporation of the chloroform extract yielded only starting material (20 mg).

ACETYLATION OF 6b

Compound 6b (4 mg) was dissolved in a mixture (1:1) of pyridine-acetic anhydride (0.3 ml). After eight hours the solvent was evaporated in a stream of nitrogen and the residue dissolved in water. The aqueous solution was made basic with aqueous sodium bicarbonate (5%) and extracted with chloroform. Evaporation of the chloroform extract yielded the N-acetyl derivative of 6b. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 1620 cm^{-1}; Bohlmann bands.

HYDROBORATION OF ANHYDROLYCOCERNUINE: PREPARATION OF LYCOALLOCERNUINE (13).

Diborane was generated in a three necked flask provided with a dropping funnel, an inlet for a stream of nitrogen and a connecting tube. Sodium borohydride (50 mg) dissolved in diglyme (10 ml) (dried over lithium aluminum hydride) was added dropwise to a mixture of freshly distilled borontrifluorideetherate (3 ml) in diglyme (5 ml) during a fifteen minute period.

By means of a stream of nitrogen the diborane was carried over to a flask (provided with a magnetic stirrer) containing anhydrolycocernuine (50 mg) dissolved in dried (over lithium aluminum hydride) tetrahydrofuran, at room temperature. After addition of diborane was complete the reaction mixture was stirred for fifteen minutes then interrupted (total reaction time thirty minutes) by cautiously adding chips of ice to the ice-cooled tetrahydrofuran solution. Vigorous evolution of gas occured. Once it had subsided the solution was poured into a flask containing aqueous sodium hydroxide (2 ml, 30%) and hydrogen peroxide (1.5 ml., 30%). This

mixture was stirred for two hours.

The organic residue isolated from this operation (45 mg) showed on t.l.c. (Al_2O_3) mostly one product, contaminated by small amounts of starting material and at least one other component. The main product was isolated by chromatography on alumina, elution with chloroform. The R_F of this product was different from that of lycocernuine (2b) and epilycocernuine (2c).

Infrared spectrum: $\gamma_{max.}^{CCl_4}$ 3550, 1640 cm^{-1}, 1420 cm^{-1}. Calculated for $C_{16}H_{26}O_2N_2$, molecular wt., 278. Found, molecular wt. 278. The product was not further characterized but used to prepare the acetyl derivative.

ACETYL DERIVATIVE OF LYCOALLOCERNUINE

Lycoallocernuine (23 mg) was dissolved in a mixture of pyridine-acetic anhydride (1:1). The reaction was worked up in the usual manner leading to the isolation of the crude O-acetyl derivative. The product was purified by chromatography on alumina, the bulk of the acetylated derivative being eluted with ether. Infrared spectrum: $\gamma_{max.}^{CCl_4}$ 1730, 1630 1415 cm^{-1}. Nuclear magnetic resonance spectrum: τ 5.03 (1H, quartet, splitting 2 and 9 cps), 5.34 (1H, half-height width 6 cps), 7.90 (3H, singlet), 9.10 (3H, doublet, splitting 5.8 cps).

DEUTERIUM LABELLING

a) Deoxylycocernuine-1,1-d_2

Lycocernuine (10 mg) was dissolved in dried tetrahydrofuran, and lithium aluminum deuteride (10 mg) was added to this solution. This mixture was refluxed for twenty hours. Excess deuteride was destroyed in the usual manner, the granulous precipitate was filtered off, and the filtrate

dried and evaporated. Recrystallization of the residue from acetone afforded dideuterodeoxylycocernuine (mass spectrometry, m.p. and t.l.c.).

b) Lycocernuine-12-d_1

Dehydrolycocernuine (30 mg) was dissolved in methanol (6 ml). To this solution sodium borodeuteride (20 mg) and sodium carbonate (30 mg) were added. The mixture was stirred for twelve hours at room temperature. The reaction was worked up in the usual manner. Recrystallization from acetone afforded monodeuterolycocernuine (mass spectrometry, t.l.c., and m.p.).

c) Monodeutero-d_7-derivative of O-acetyllycocernuine

Oxazolidine (10) perchlorate salt (40 mg) was dissolved in methanol, and sodium borodeuteride (30 mg) added to this solution. The mixture was left at room temperature for ten hours, the solvent was then evaporated and the residue dissolved in water and extracted with chloroform. Evaporation of the chloform extract afforded a product with R_F identical to that of lycocernuine. This product was dissolved in a mixture of pyridine-acetic anhydride (1:1) (6 ml) and left overnight at room temperature. The usual work up of this solution afforded crude monodeutero-O-acetyl-lycocernuine (mass spectrometry and t.l.c.). The product was purified by chromatography on alumina. Nuclear magnetic resonance spectrum: τ 4.5 (1H, quartet, splitting 12 and 2 cps), 5.1 (1H, poorly resolved multiplet, half-height width 5 cps), 7.95 (3H, singlet), 9.18 (3H, doublet, splitting 6 cps).

DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY OF LYCOCERNUINE (2c)

The method used is the one described by A. Horeau et al.[71,72].

(i) Preparation of α-phenylbutyric acid anhydride.

α-Phenylbutyric acid (50 g) was refluxed for five hours with acetic anhydride (125 ml). The excess anhydride and acetic acid was distilled off at atmospheric pressure (110° - 130° C) then completely eliminated with the water-pump.

The residue was distilled under vacuum (0.1 mm/Hg), using a short column, in a 120° - 130° C range. The viscous yellowish liquid was redistilled once more to get a colorless, viscous liquid with the spectral characteristics that could be expected from the α-phenylbutyric anhydride.

Infrared spectrum (liquid film); No OH absorption; 1815 cm^{-1} and 1750 cm^{-1} (C=O); 1050 cm^{-1} (C-O) 300 - 3100 cm^{-1} (various bands, =C-H); 1615 cm^{-1}; 1505 cm^{-1} (C=C) 710 cm^{-1} and 755 cm^{-1} (C-H, out-of-plane).

N.m.r. spectrum ($CHCl_3$): τ 2.80 (5H, singlet); 6.55 (1H, triplet, J = 7 cps); 8.10 (2H, octet, J = 7 c.p.s.); 9.20 (3H, triplet, J = 7 c.p.s.).

(ii) Determination of the absolute stereochemistry:

A stock solution was prepared with the α-phenyl butyric anhydride in pyridine: α-phenyl butyric anhydride (1.8165 g) was made up to approximately 10 ml. Total weight: 10.1225 g (1 gram solution: 0.180 g α-phenylbutyric anhydride).

A portion (0.4632 g) of the above stock solution was used to esterify lycocernuine (28.9 mg). This mixture was left standing for twenty hours. After this time one drop of water was added and the mixture was warmed

in the steam-bath for thirty minutes, then poured into an Erlenmeyer containing water (2 ml) and benzene (3 ml). This solution was titrated with aqueous sodium hydroxide (0.1 N, f: 1.13) using phenolphthaleine as the indocator. Amount of sodium hydroxide consumed: 4.00 cc.

The organic layer was separated and the extraction repeated with chloroform and again with benzene.

The aqueous layer was acidified with aqueous hydrochloric acid (~6n) and extracted with benzene (8 ml). The benzene extract (8 cc) was dried and used to obtain the optical rotation.

Reading at ν_{589}: + 0.075° (α).

CALCULATIONS

Symbols and data:

Weight of the hydroxylic compound: a

Weight of α-phenylbutyric anhydride used: b

Molecular weight of the hydroxylic compound: M

No. of c.c. of NaOH 0.1 N: n

Molecular weight of the α-phenylbutyric anhydride: 310

Molecular weight of α-phenylbutyric acid: 164

Specific rotation of α-phenylbutyric acid: ±96.5°

Esterification yield (E) will be given by:

$$E = \frac{Mb}{155a} - \frac{nM}{a}$$

Optical yield (defined as the ratio between the experimental reading (α) and the reading that one would expect (α') if the unsymmetric esterification would have taken place in a genuinely specific way and

the esterification had been complete): Optical yield $\frac{\alpha}{\alpha' E}$

These calculations applied to the experiment described above give:

$$E = \frac{278 \times 78.7}{155 \times 28.9} - \frac{0.452 \times 278}{28.9} = 0.49 \text{ or } 49\%$$

Optical yield: $\frac{0.075}{0.205 \times 49} 100 = 0.74$ or 74%

This experiment repreated with dihydrodeoxylycocernuine gave consistent results: E = 52%; optical yield 13%.

It was also applied to dihydrolycopodine and epidihydrolycopodine, a positive and negative reading being obtained respectively. These results are consistent with the known absolute stereochemistry of lycopodine.

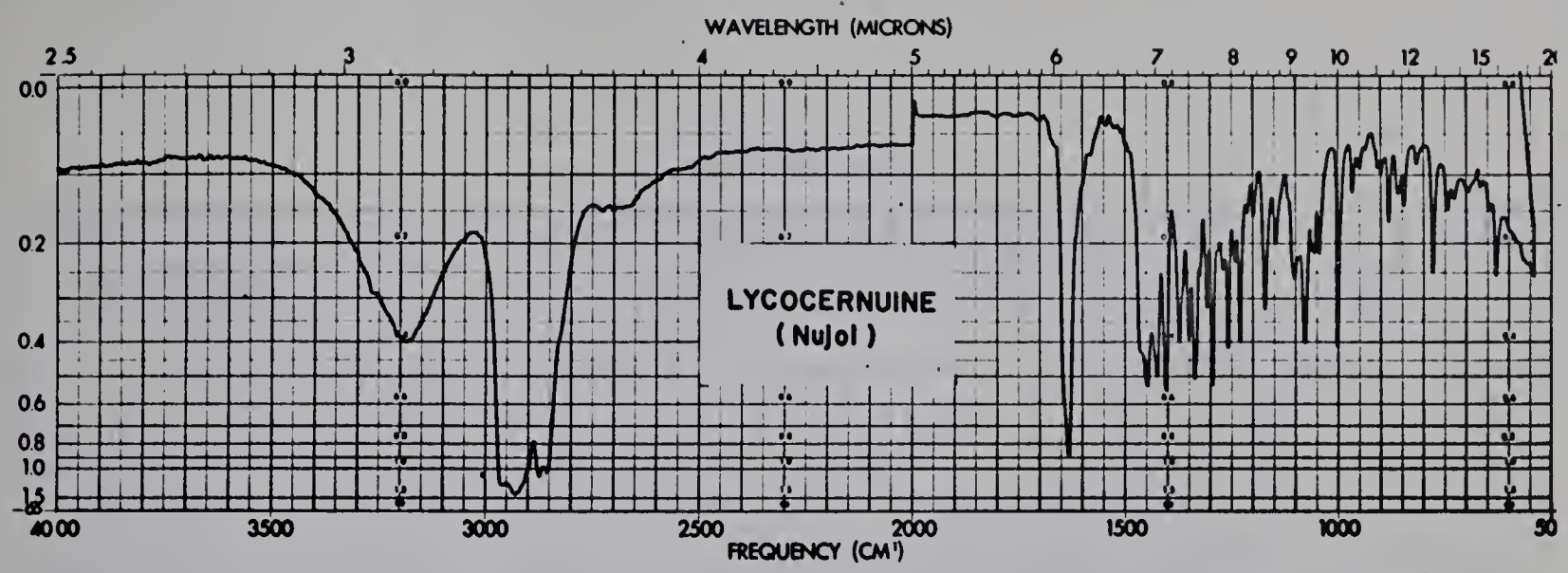
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
0.0
0.2
0.4
0.6
0.8
1.0
1.5
LYCOCERNUINE
(Nujol)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM⁻¹)

FIG. 1

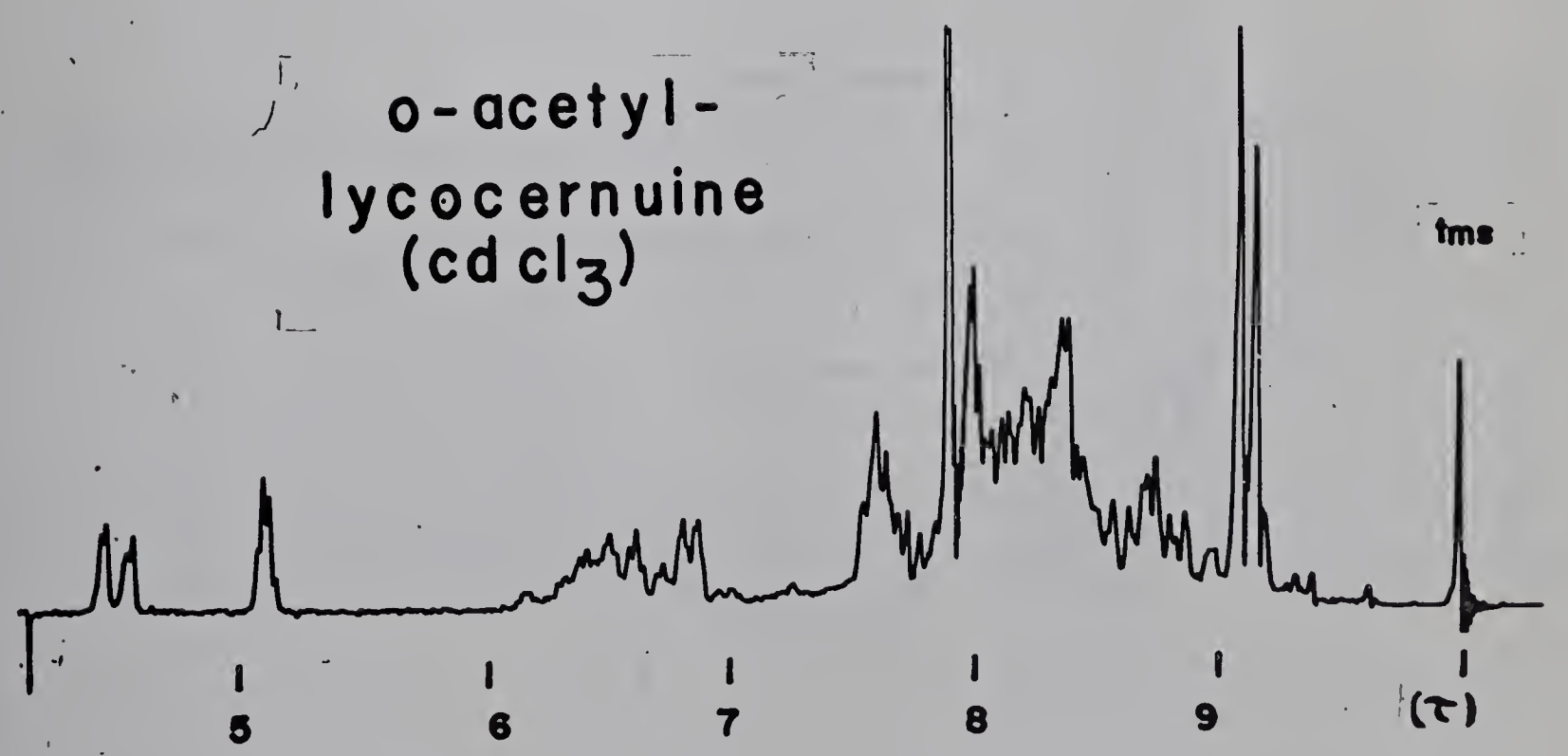
o-acetyl-
lycocernuine
(cd cl3)
tms
5
6
7
8
9
(τ)

FIG. 2

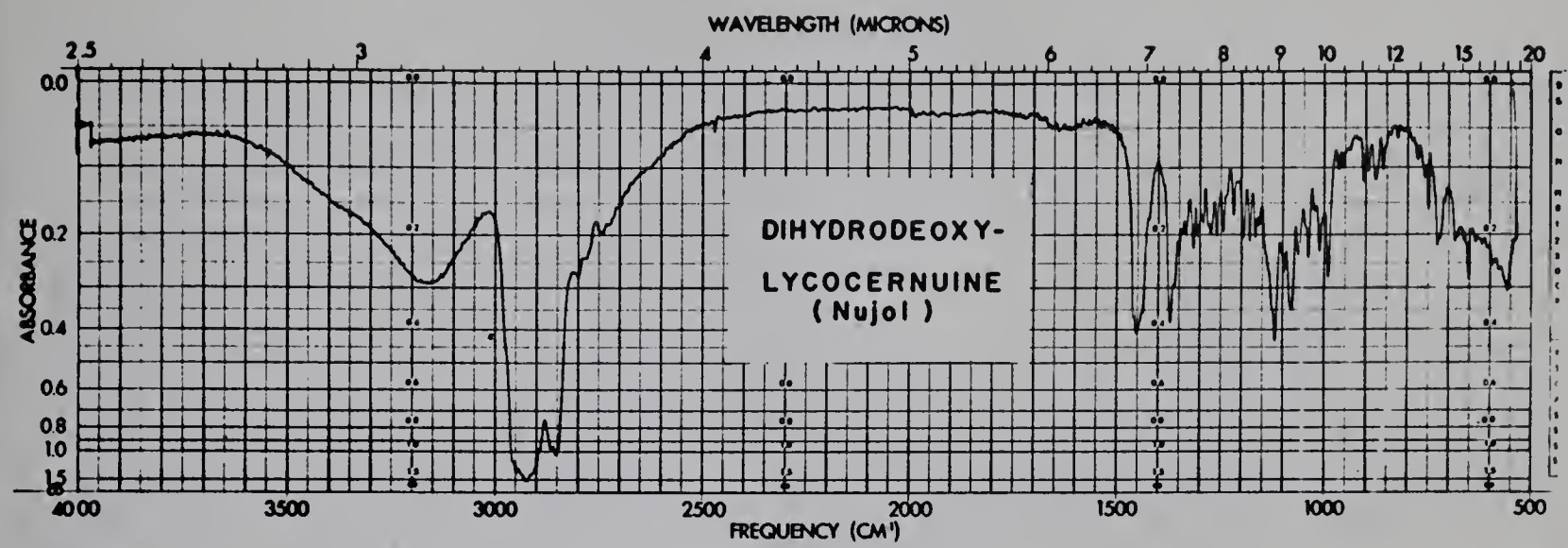
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
20
ABSORBANCE
0.0
0.2
0.4
0.6
0.8
1.0
1.5
DIHYDRODEOXY-
LYCOCERNUINE
(Nujol)
4000
3500
3000
2500
2000
1500
1000
500
FREQUENCY (CM⁻¹)

FIG. 3

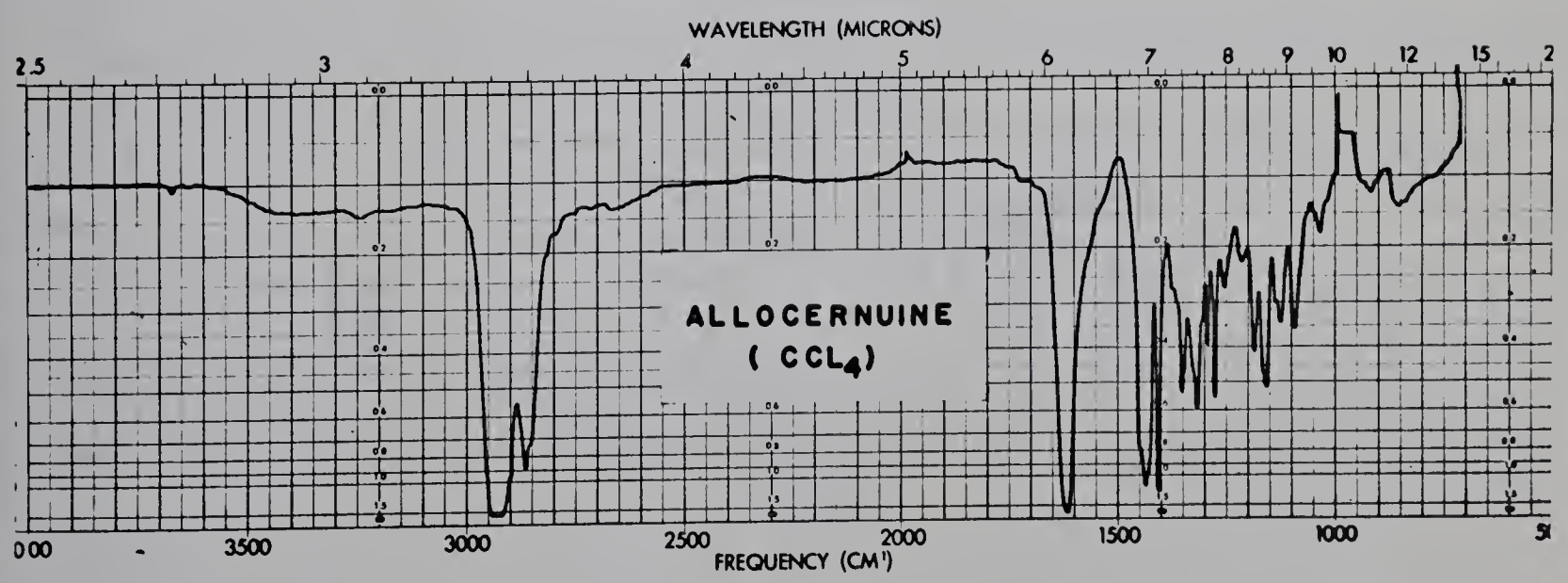
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
ALLOCERNUINE
(CCL4)
3500
3000
2500
2000
1500
1000
FREQUENCY (CM⁻¹)

FIG. 4

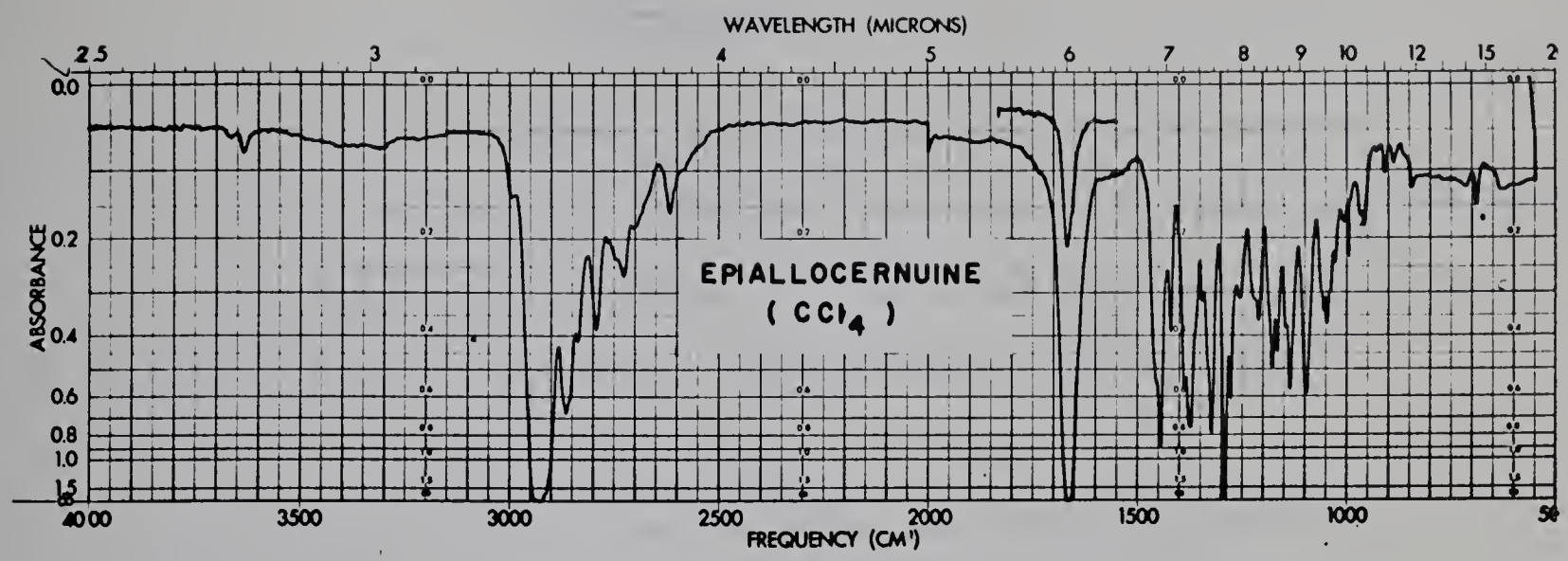
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
ABSORBANCE
0.0
0.2
0.4
0.6
0.8
1.0
1.5
EPIALLOCERNUINE
(CCl4)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM⁻¹)

FIG. 5

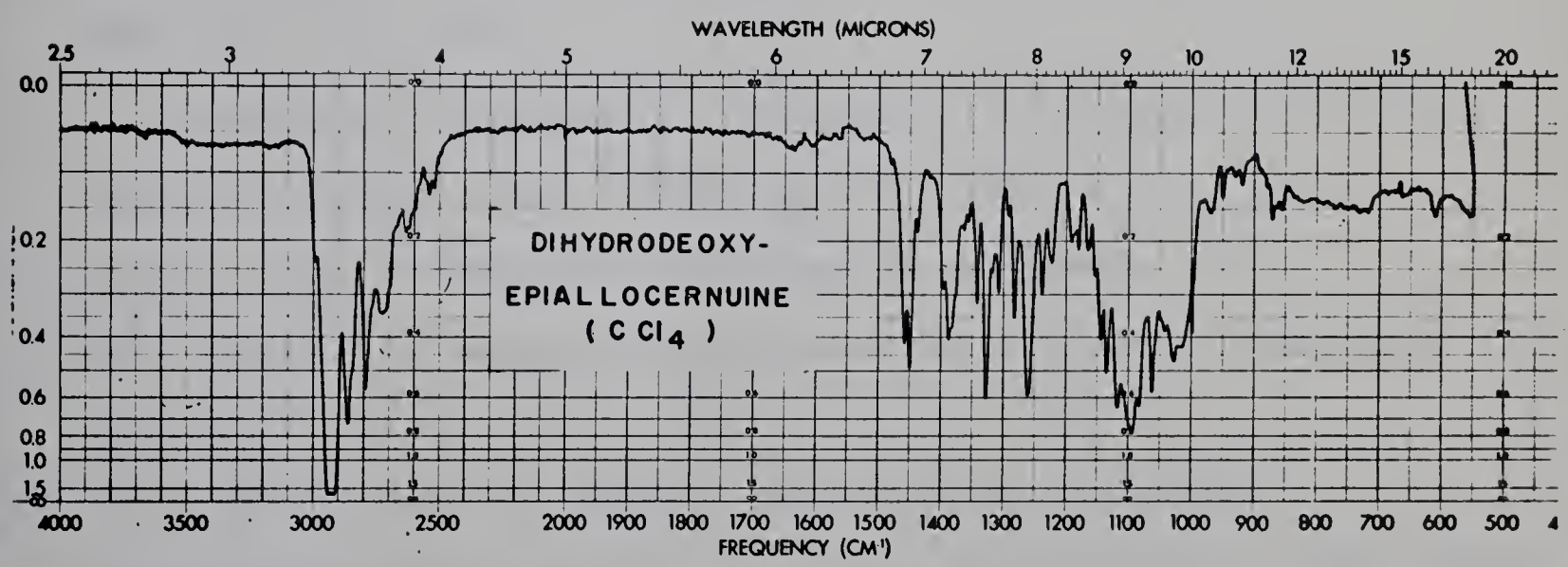
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
20
0.0
0.2
0.4
0.6
0.8
1.0
1.5
DIHYDRODEOXY-
EPIALLOCERNUINE
(CCl4)
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
FREQUENCY (CM⁻¹)

FIG. 6

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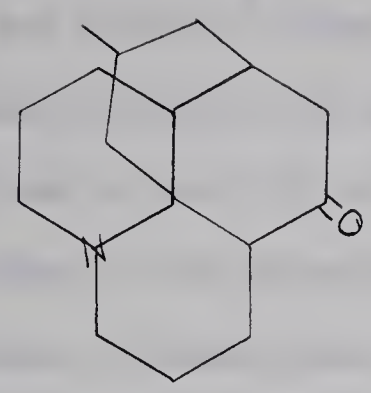
The alkaloids of Lycopodium alopecuroides L.

- A preliminary survey -

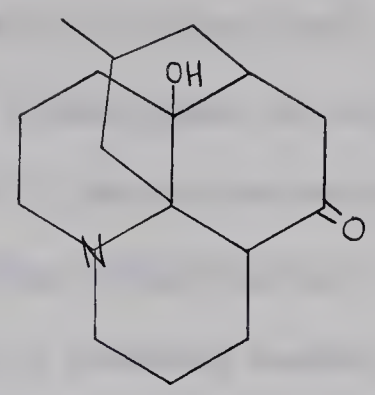
Despite the fact that well over two hundred different species of the genus Lycopodium are known, only a very small number have been investigated in detail. The presence of alkaloids in Lycopodium alopecuroides L. had been noted before[1], but no attempt had been made to isolate and study individual components. This investigation has led to the isolation of seven compounds, three of which have not been previously recorded in the literature.

The crude basic material obtained from Lycopodium alopecuroides was subjected to chromatography on alumina. Elution with benzene furnished lycopodine (1), major alkaloid present. Elution with ether yielded a mixture of two alkaloids of similar R_F value. Crystallization from acetone afforded one of the components in pure state. This component, the properties of which (see below) do not correspond to any of the previously known Lycopodium alkaloids has been named alopecurine. Concentration of the mother liquors from this crystallization furnished crystals of lycodoline[2] (2), the identity of which was established by means of its infrared spectrum, melting point and mixed melting point.

The next component, eluted with chloroform, was clavolonine[3] (3), identified by its infrared spectrum and t.l.c. behaviour.



1.



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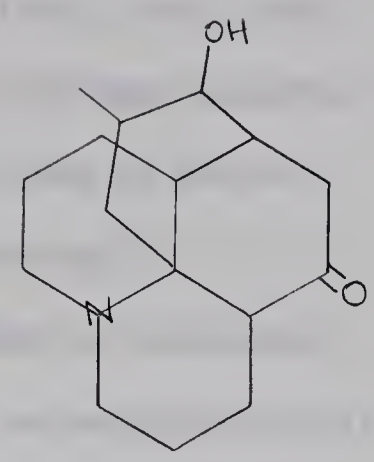


3.

Further elution with chloroform yielded the second previously unreported compound, which we have named alopecuridine. A considerable amount of the crude alkaloid is made up of compounds of greater polarity (i.e. lower R_F value) than alopecuridine. Despite considerable effort to separate these products into individual components all attempts have met with failure, except for the isolation in one instance of a small amount of a component which we shall refer to as "base 16".

Two of the new bases, alopecurine and alopecuridine, have been characterized and some of their reactions will be reported below. Passing mention will be made of the interesting properties of "base 16".

The first one of these bases, alopecurine, is a crystalline compound, m.p. 244 - 245°, (methiodide, m.p. 250°), to which the molecular formula $C_{23}H_{29}O_3N$ is assigned on the basis of analytical and mass spectral data. The ultraviolet, infrared and mass spectra indicate the presence of a benzoyloxy group in alopecurine. Thus, the ultraviolet spectrum showed typical benzoate absorption[4] at 230, 272 and 280 mμ.; the infrared spectrum

of alopecurine (Nujol)(see Fig. 1) exhibited bands at 1705, 1595, 1580, 1270 and 1110 cm^{-1} characteristic of benzoates[5], while the mass spectrum (molecular weight 367) showed strong peaks at m/e 245 (loss of benzoic acid) and at m/e 122, 105 and 77 due to the benzoate portion[6].

Most of the known Lycopodium alkaloids are C_{16} bases or acetylated C_{16} bases. Alopecurine, with 23 carbons, seemed to be an exception until the presence of a C_7 benzoate group was determined. Alopecurine is the first benzoylated Lycopodium alkaloid to be reported.

The benzoyloxy group accounts for two oxygens in the molecule. The third oxygen of alopecurine is present as a hydroxyl group as shown by the presence of OH absorption in the infrared spectrum and by the formation of a mono-O-acetyl derivative on acetylation. Both alcoholic functions present in alopecurine seem to be secondary in nature. In the nuclear magnetic resonance spectrum of alopecurine we encounter a low field proton at τ 4.65, probably associated with the benzoyloxy group. Another one-proton signal at τ 6.0 may be assigned to the proton geminal to the hydroxyl group. In agreement with this assignment, this last signal is shifted downfield (τ 4.8) upon formation of the O-acetyl derivative. Furthermore the signal is not present in the n.m.r. spectrum of the dehydroderivative of alopecurine, obtained by oxidation (Jones' reagent) of the hydroxyl group to a keto group. Lack of NH absorption in the infrared spectrum of O-acetylalopecurine indicates that the nitrogen is tertiary.

Alkaline hydrolysis of alopecurine yielded benzoic acid and debenzoyl-

alopecurine*, $C_{16}H_{25}O_2N$, m.p. 230 - 232°, which does not seem to be identical with any Lycopodium alkaloid or derivative of the same molecular formula.

Alopecurine is recovered unchanged on attempted hydrogenation at atmospheric pressure in methanol with palladium-charcoal as the catalyst. Hydrogenation at 50 psi in methanol solution in the presence of Adam's catalyst leads to the uptake of three moles of hydrogen as evidenced by an increase of six mass units in the molecular weight of the O-acetyl derivative of the hydrogenation product. This result can be best explained on the basis of reduction of the aromatic ring. This interpretation is confirmed by the absence of the characteristic benzoate absorption in the ultraviolet spectrum of the hydrogenation product. It also indicates that the C_{16} portion of the molecule does not contain a double bond, unless it is extremely hindered.

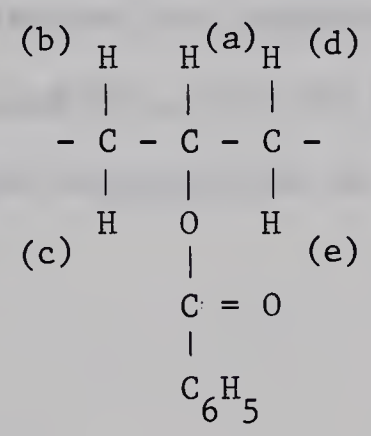
Whether debenzoylalopecurine is a pentacyclic alkaloid (as required by its molecular formula) or it contains a tetracyclic skeleton with a further unsaturation (e. g., a tetrasubstituted olefin) cannot be established with the information available. It is hoped that Raman spectroscopy will be able to settle this point. In either case, these requirements (a pentacyclic skeleton or a tetrasubstituted double bond)[7] are sufficient to eliminate the possibility of a lycopodine type of structure for alopecurine and all the information at hand suggests that again we are dealing with

---

* Debenzoylalopecurine has also been isolated in small amounts directly from the plant extract. B. Altenkirk. Private communication. Whether it occurs as such or is formed by hydrolysis of alopecurine during the isolation procedure is not known.

a novel type of alkaloid.

The n.m.r. spectrum of alopecurine (see Fig. 2) is very informative and serves to establish the environment of the benzoyloxy group. A series of one-proton quartets at τ 6.25 (splitting 15.5 and 7.5 cps), 6.9 (splitting 15 and 10 cps), 7.25 (splitting 15.5 and 3.5 cps) and 8.4 (splitting 15 and 6 cps) are all coupled to the proton geminal to the benzoyloxy group. This is best rationalized if we assume that the four protons belong to two methylenic carbons <u>alpha</u> to the carbon atom bearing the benzoyloxy group. The situation is schematically represented below [A]



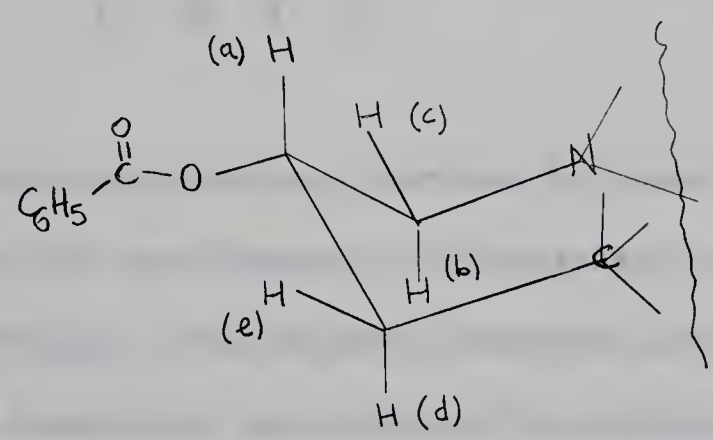
[A]

Each of the signals due to protons (b), (c), (d) and (e) exhibits a large coupling constant (~15 cps) which must be a geminal coupling[8]; the second splitting of each of the quartets ranges from 3.5 - 10 cps and must be due to the coupling with proton (a). This proton must exist in the axial configuration in order to be able to account for the larger splittings[9] (7.5 and 10 cps) present in two of the quartet signals.

The partial structure [A] above is fully supported by double irradiation experiments carried out with alopecurine. For a better understanding we will assign the quartet at τ 6.25 to proton (b), 7.25 to (c), 6.9 to (d) and the one at 8.4 to proton (e). On simultaneous irradiation of proton (a) at τ 4.65 the four quartets collapse to doublets with splittings

of 15.5 cps [(b) and (c)] and 15 cps [(d) and (e)]. Simultaneous irradiation of proton (b) at τ 6.25 causes collapse of the (c) quartet to a doublet (splitting 3.5 cps), while the other signals [(d) and (e)] are unaffected; at the same time the complex multiplet due to proton (a) is simplified. Analogously, simultaneous irradiation of proton (d) at τ 6.9 brings about collapse of the (e) quartet to a doublet (splitting 6 cps), leaving unchanged the signals due to protons (b) and (c) and simplifying that due to proton (a). Analogous experiments were carried out with the O-acetyl derivative of alopecurine and confirmed these results.

Taking into consideration the magnitude of the coupling constants between proton (a), in one side, and (b), (c), (d) and (e) in the other, one can assign equatorial or axial configuration to these protons in the manner indicated below: [B]



[B]

Since protons (b), (c), (d) and (e) do not exhibit any further splitting they must be flanked either by tetrasubstituted carbons or the nitrogen atom and a tetrasubstituted carbon. The low chemical shift of proton (b) favors this second possibility, which has in fact been incorporated into partial structure [B].

The environment of the secondary hydroxyl group in alopecurine is revealed by results obtained with the anhydroderivative of alopecurine. Anhydroalopecurine was prepared by dehydration of alopecurine with thionyl chloride in benzene. The n.m.r. spectrum of the anhydro compound shows signals for two olefinic protons at τ 4.5 and 4.65. The one proton signal at τ 4.5 appears as a doublet (splitting 9 cps) while the proton at τ 4.65 is a quartet (splitting 9 and 2 cps). This result indicates the following part structure:

```
          H   H   H
          |   |   |
    - C - C = C - C -
                    |
```

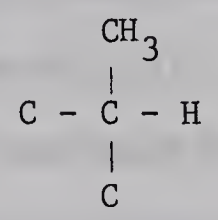
hence alopecurine must possess either one of the systems schematized below [C]:

```
                                                                  [C]
        H   H   H   (C)                          (C)  H   H   H
        |   |   |   |                            |   |   |   |
(C) - C - C - C - C - (C)   or   (C) - C - C - C - C - (C)
        |   |   |   |                            |   |   |   |
      (C)  OH  H  (C)                          (C)  OH  H  (C)
```

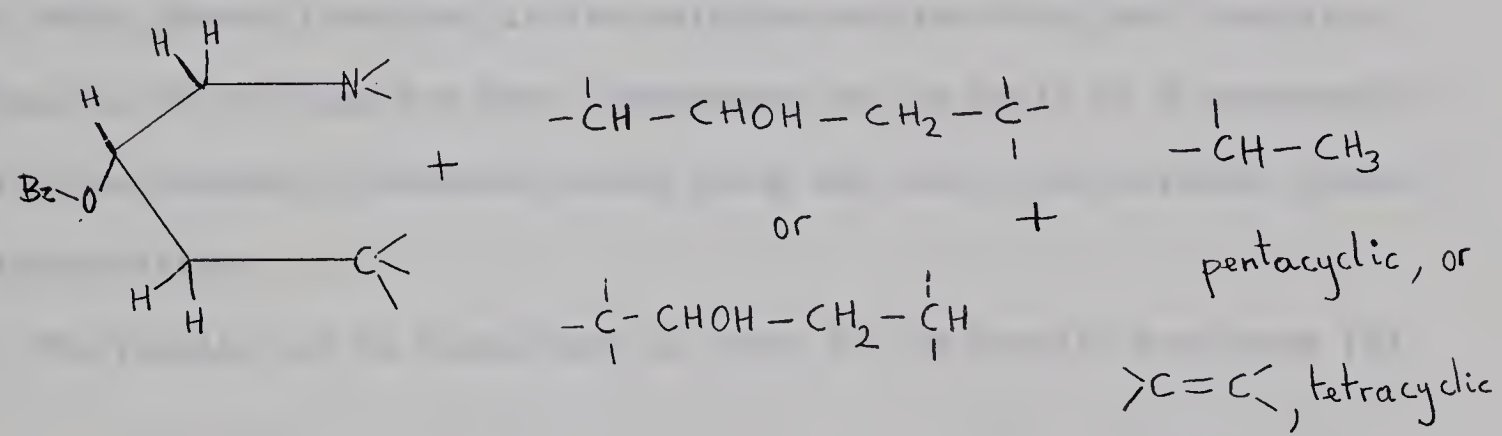
Dehydroalopecurine, prepared by Jones' oxidation of alopecurine exhibited the spectroscopic characteristic expected from the ketoderivative of alopecurine. The infrared spectrum (chloroform solution) does not show NH or OH absorption and exhibits two carbonyl bands at 1710 cm^{-1} and 1685 cm^{-1}. The n.m.r. spectrum of dehydroalopecurine retains the signals due to protons (a), (b), (c), (d) and (e) (part structure [B]). The signal at τ 6.0 in alopecurine and τ 4.8 in O-acetylalopecurine assigned to the proton geminal to the hydroxyl is no longer present.

A deuterium exchange carried out with the dehydroderivative of alopecurine could help to decide between the two partial structures in [C].

Finally, alopecurine and all its derivatives display a three proton doublet at τ 9.0, indicative of the grouping:



The following part structure may thus be written for alopecurine:

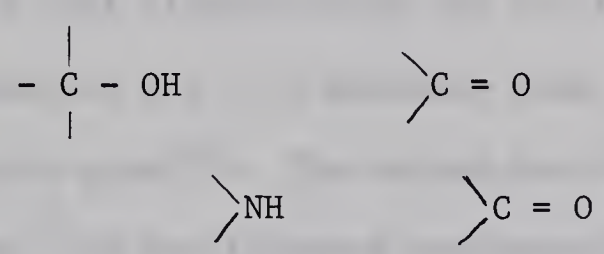


The second new alkaloid, alopecuridine, is also a crystalline compound, m.p. 171 - 172°. Analytical and mass spectral data indicated the molecular formula $C_{16}H_{25}O_3N$ for alopecuridine. The infrared spectrum of this compound is unusual in that the spectrum obtained in solution differs considerably from the Nujol mull spectrum. The spectrum determined in chloroform showed strong OH absorption at 3575 cm^{-1} and weak absorption at 3330 cm^{-1} (NH). The carbonyl region exhibited three bands, a strong band at 1740 cm^{-1}, medium intensity absorption at 1690 cm^{-1} and weak absorption at 1595 cm^{-1} (see Fig. 3). The spectrum of alopecurine (Nujol mull) (see Fig. 4) crystallized from acetone showed a series of

bands in the OH, NH region as well as carbonyl absorption at 1730 cm^{-1} (strong) and 1640 cm^{-1} (medium intensity). The Nujol spectrum of a sublimed sample of alopecuridine showed a sharp band at 3480 cm^{-1} and a single carbonyl band at 1710 cm^{-1} (see Fig. 5). Both of these crystalline forms exhibit the solution spectrum mentioned above. The infrared spectrum of the perchlorate (Nujol mull) also showed a single carbonyl band at 1750 cm^{-1}.

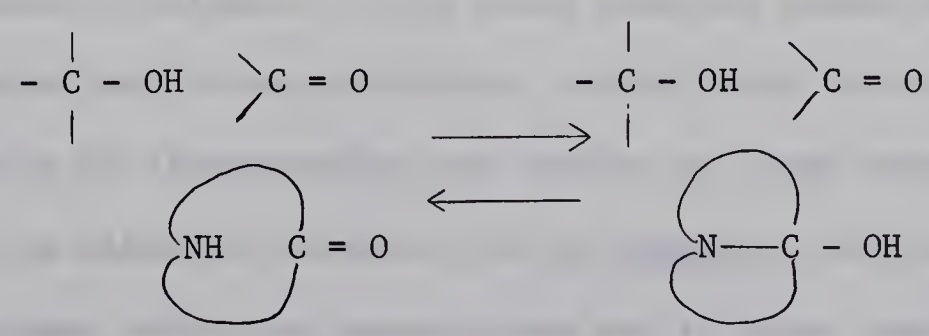
Acetylation of alopecuridine yielded the N-acetyl derivative, $C_{18}H_{25}O_4N$, which showed hydroxyl absorption as well as carbonyl absorption at 1740, 1685 and 1620 cm^{-1} (see Fig. 6), the number and position of the bands being almost identical in the solution and the Nujol mull spectra. The spectra of the base are best interpreted on the basis of a transannular interaction between a secondary amino group and one of two carbonyl groups in alopecuridine.

The results may be summarized in terms of the partial structure [E].



[E]

which accounts for the functional groups present in the molecule. It is postulated that an equilibrium exists between two forms schematized by means of part structures [F] and [G].



[F] [G]

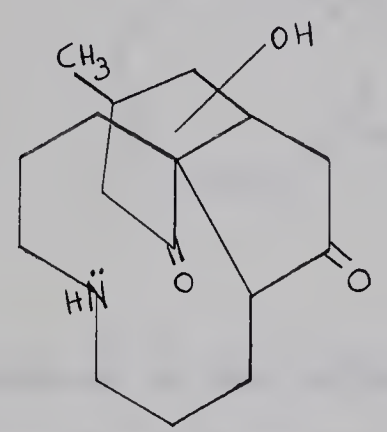
The chloroform spectrum of the base and the Nujol spectrum of the crystallized material represent form [F] or an equilibrium mixture of [F] and [G], whereas the sublimed sample must be entirely in form [G]. The perchlorate exists in form [G] but the acetyl derivative corresponds to form [F]. The fact that the non-interacting carbonyl group absorbs at different positions (e. g. 1730 cm^{-1} in the crystallized base, 1710 cm^{-1} in the sublimed base) may be due to varying degrees of intermolecular hydrogen bonding in the crystal. The possibility that one of the carbonyl groups in partial structure [E] is an aldehyde carbonyl is excluded by the fact that the nuclear magnetic resonance spectrum of N-acetylalopecuridine shows no absorption below τ 6.0. This also rules out the presence of olefinic protons. The n.m.r. spectrum does show the presence of a secondary C-methyl group as a three-proton doublet at τ 8.9 as well as the acetyl singlet at τ 7.9.

It thus appears that alopecuridine has two ketonic carbonyl groups one of which is present in a 8 - 10 membered ring which contains a trans-annular secondary amino group[10]. The second keto group gives rise to absorption at 1740 cm^{-1} in the infrared and hence may be located in a five membered ring.

The hydroxyl group is not readily acetylated and must be either tertiary or highly hindered or both. Repeated attempts to prepare the anhydro compound ended in failure. Since there does not appear to be a carbon-carbon double bond in alopecuridine, the molecule is tricyclic. Some of the properties of alopecuridine are similar to those described for fawcettimine[11], an alkaloid isolated from L. fawcetti. Alopecuridine contains one more oxygen atom than fawcettimine and it seems possible that

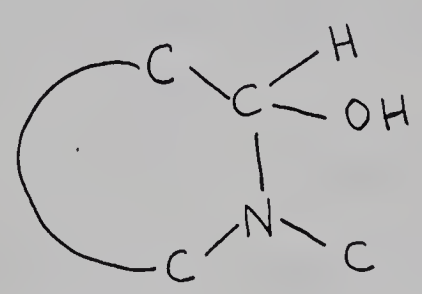
alopecuridine is a hydroxylated fawcettimine.

The publication of the structure of serratinine by Inubushi and coworkers[12] contains the suggestion that the skeleton of serratinine could be derived from lycodoline (2); an intermediate such as (b) (see page 11) accounts for many of the properties of alopecuridine and offers a reasonable working hypothesis for the structures of alopecuridine and that of fawcettimine:

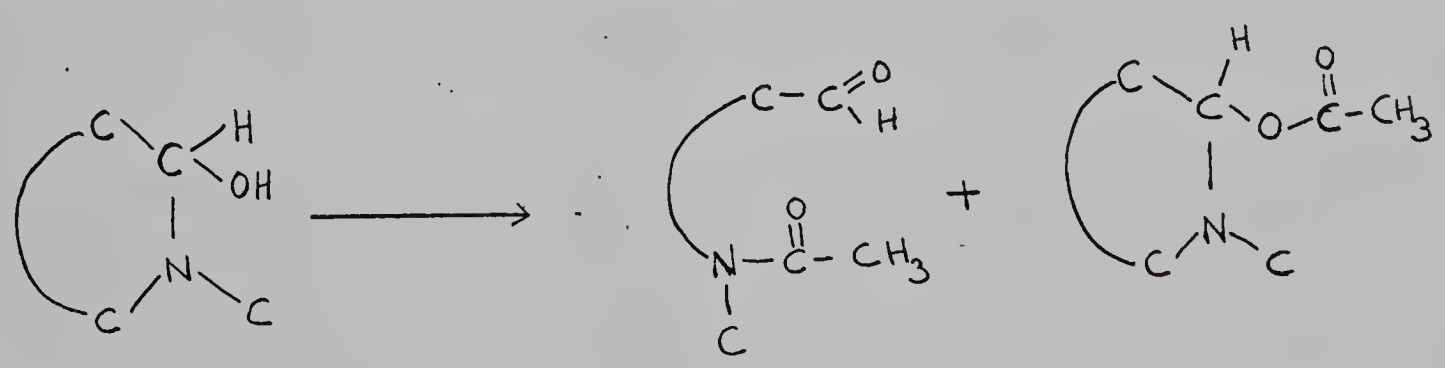


"Base 16" is a third crystalline product (m.p. 239 - 241°) isolated from the latter fractions of the chromatographic separation. The small amount of material available (24 mg) made difficult a complete characterization of this compound. The molecular formula $C_{16}H_{25}NO$ was assigned to this compound on the basis of mass spectral data. The infrared spectrum of this compound ($CHCl_3$ solution) showed OH absorption at 3630 cm^{-1} and was devoid of carbonyl absorption of any type. The compound was acetylated with pyridine-acetic anhydride and two products were obtained. The products had very similar R_F values and could not be separated chromatographically. An infrared spectrum obtained with this mixture showed bands in the carbonyl region at 1715 and 1620 cm^{-1} (strong) and weak absorption at 2720 cm^{-1}.

An n.m.r. spectrum obtained with the same mixture showed singlets at τ 7.92 and 7.94 respectively, as well as a doublet at τ 9.1 and a very low-field signal at τ 0.25. The signal at τ 0.25 integrates for less than one proton and the two singlets at τ 7.92 and 7.94 together integrate for three protons, taking the methyl group at τ 9.1 as the standard. The information provided by the spectroscopic data suggests the presence of the system



in "base 16". Acetylation of the carbinolamine would then give rise to a mixture of the O-acetyl and N-acetyl derivatives with simultaneous creation of an aldehydic function.



When the mixture of acetylated products was reduced with sodium borohydride and the infrared spectrum of the product determined it was found that the intensity of the carbonyl band at 1720 cm^{-1} was considerably diminished while the band at 1620 cm^{-1} was unaffected by this treatment. The product exhibited OH absorption at 3610 cm^{-1}, but absorption in the 2600 -

2800 cm^{-1} region was no longer present.

Since no unsaturation seems to be present in "base 16" a pentacyclic system is indicated. Lack of material prevented further work on this substance, but it is interesting to note that two of the new alkaloids from this species appear to have pentacyclic frameworks.

Experimental

Infrared spectra were recorded on a Perkin-Elmer Model 421 dual grating infrared spectrophotometer or a Perkin-Elmer Model 337 grating infrared spectrophotometer.

Nuclear magnetic resonance spectra were measured in deutero-chloroform solution, unless otherwise specified, using a Varian Associates Model A-60 spectrometer or a Varian Associates Model HR-100 spectrometer with tetramethylsilane as an internal standard.

Mass spectra were determined on an A.E.I. Model MS-2H or an A.E.I. Model MS-9 mass spectrometer, with heated inlet (170° - 200°); electron energy 70 e.v.

Melting points were determined on a hot-stage Fischer-Johns melting point apparatus and are uncorrected.

Alumina, unless otherwise specified, means basic alumina of activity III - IV (Brockman scale).

Research Specialty Company aluminum oxide G was used for thin layer chromatography.

Microanalysis are by F. Pascher, Bonn, Germany; or by C. Daessle, Montreal, Quebec.

Separation of the alkaloids. A benzene solution of the crude alkaloid (4.5 g) obtained from the Smith, Kline, French Laboratories, Philadelphia, was placed on a column of basic alumina (150 g, activity III) and eluted in fifteen fractions as follows:

Fraction	Eluant	Volume (ml)	Weight of fraction (g)
1	benzene	150	0.02
2	benzene	150	0.06
3	benzene	150	0.16
4	benzene	150	0.15
5	benzene:ether (4:1)	150	0.16
6	benzene:ether (4:1)	150	0.23
7	ether	150	0.10
8	ether	150	0.43
9	chloroform	150	
10	chloroform	150	0.31
11	chloroform	150	
12	chloroform-methanol (49:1)	200	0.25
13	chloroform-methanol (44:1)	200	1.06
14	chloroform-methanol (24:1)	200	0.91
15	chloroform-methanol (9:1)	300	0.29

Fractions 1 and 2 contained non-basic material and were not further investigated. Fraction 3 showed two spots on t.l.c.; the more polar

of these two components was identified as lycopodine*. Fractions 4, 5 and 6 contained mainly lycopodine. Fraction 7 contained five components none of which could be isolated in pure form. Fraction 8 contained three components, the least polar probably lycopodine (t.l.c.); the material from this fraction was dissolved in hot acetone. On cooling, colorless crystals of alopecurine (0.10 g) separated which, after recrystallization from acetone, melted at 244 - 245°.

Analysis. Calculated for $C_{23}H_{29}O_3N$: C, 76.17; H, 7.95; N, 3.81% Found: C, 75.52; H, 7.94; N, 3.67%. Molecular weight 367 (mass spectrometry).

Infrared spectrum: $\gamma_{max.}^{Nujol}$ 1705, 1595, 1580, 1270 and 1110 cm^{-1}; broad absorption at 3100 cm^{-1} (see Fig. 1). Ultraviolet spectrum: $\gamma_{max.}^{EtOH}$ 230, 272 and 280 mμ. Nuclear magnetic resonance spectrum: (see Fig. 2) τ 4.65 (1H, multiplet $w_{1/2}$ 15 cps), τ 6.0 (1H, doublet, splitting 8 cps), 6.25 (1H, quartet, splitting 1.5 and 7.5), 6.9 (1H, quartet, splitting 15 and 10 cps) 7.25 (1H, quartet, splitting 15.5 and 3.5 cps); 8.4 (1H, quartet, splitting 15 and 6 cps), 9.05 (3H, doublet, splitting 6 cps).

Concentration of the acetone mother liquors above yielded crystalline lycodoline, m.p. 179 - 180°, identical (t.l.c., i. r. spectrum, mixed m.p.) with an authentic sample. Fractions 9, 10 and 11 contained mostly lycodoline plus another minor component which showed the same R_F as clavolonine.

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* Subsequent to this work, the less polar component present in fraction 3 was obtained in pure form by subjecting the mixture of the two components to chromatography on a dry-packed alumina column[13]. The material was identified as anhydrolycodoline[2]. B. Altenkirk. Private communication

Clavolonine was also present in fraction 12. Fraction 12 was dissolved in hot acetone. Cooling of this acetone solution yielded clavolonine slightly contaminated by another component. Repeated recrystallization from hot acetone afforded pure clavolonine. Its identity was established by comparison with the infrared spectrum of an authentic sample. The mother liquor from fraction 12 and fractions 13 and 14 contained alopecuridine, which crystallized from acetone-ether solutions of these fractions. A total of 0.75 g of alopecuridine, which melted at 171 - 172°, was obtained in this way. Further small amounts of alopecuridine were obtained by rechromatography of the mother liquors, but the bulk of the remaining material, which was a dark brown resin which did not move from the origin on t.l.c. (using chloroform plus a few drops of methanol as the solvent system), could not be separated into pure components*.

Analysis. Calculated for $C_{16}H_{25}O_3N$: C, 68.79; H, 9.02; N, 5.01%. Found: C, 68.63; H, 8.88; N, 5.31%. Molecular weight, 279 (mass spectrometry). The mass spectrum showed intense peaks (percentage of base peak in brackets) at m/e 279 (3), 223 (26), 220 (21), 165 (10), 150 (100), 127 (97), 98 (69) and 97 (47). The n.m.r. spectrum (pyridine solution) showed a doublet at τ 9.12 ($CH-CH_3$) as the only cleanly resolved peak. The infrared spectrum is fully discussed in the text.

In one instance crystallization from acetone of material obtained in the most polar fraction afforded a crystalline material, m.p. 239 - 241° ("Base 16").

Calculated for $C_{16}H_{25}NO$, molecular wt., 247.1936. Found: 247.1932.

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* See below. "Base 16".

Base peak at m/e 232. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$ 3630 cm^{-1}.

O-ACETYL ALOPECURINE

Alopecurine (50 mg) was dissolved in acetic anhydride-pyridine (6 ml, 1:1) and the solution kept at room temperature for 24 hours. The solvents were then removed at the pump, the residue dissolved in chloroform and the resulting solution washed first with sodium bicarbonate solution, then with water. Evaporation of the chloroform left a glassy material which could not be crystallized but which yielded a crystalline perchlorate, m.p. > 300°, from aqueous acetone.

Analysis. Calculated for $C_{25}H_{31}O_4N \cdot HClO_4$: C, 58.87; H, 6.32%. Found: C, 59.59; H, 6.11%.

Infrared spectrum: $\gamma_{max.}^{Nujol}$ 3175, 1725, 1700, 1595, 1580, 1500, 1275, 1250 and 1100 cm^{-1}. The infrared spectrum of the free base showed no OH or NH absorption.

Nuclear magnetic resonance spectrum: τ 4.6 (1H, multiplet), 4.8 (1H, doublet with further structure), 7.9 (3H, singlet), 9.0 (3H, doublet, splitting 6 cps). Aromatic absorption at τ 1.9 and 2.5.

DEBENZOYL ALOPECURINE

Alopecurine (100 mg) was dissolved in alcohol (9 ml) and 4% aqueous KOH (6 ml) added. The resulting solution was refluxed for one and a half hours, then concentrated at the pump, diluted with water and extracted with chloroform. Evaporation of the chloroform left a solid (50 mg) which was purified by sublimation and then crystallization from acetone. The debenzoylalopecurine thus obtained melted at 230 - 232°. Infrared absorp-

tion: $\gamma_{max.}^{Nujol}$ 3350 cm^{-1} (broad OH absorption). No bands in the 1500 to 1800 cm^{-1} region. The mass spectrum showed a parent peak at m/e 263 corresponding to $C_{16}H_{25}O_2N$ and a base peak at m/e 216.

Acidification of the aqueous hydrolysis solution and extraction with chloroform afforded benzoic acid (13 mg) identical in melting point and infrared spectrum with an authentic sample.

DEHYDROALOPECURINE

Alopecurine (40 mg) was dissolved in purified acetone. This solution was cooled in ice and Jones' reagent (five drops) was added at short intervals. The acetone was evaporated and the residue dissolved in water. This aqueous solution was extracted with chloroform. Evaporation of the chloroform extract afforded dehydroalopecurine (30 mg). The product was further purified by sublimation at 170 - 180°/0.1 mm. Infrared spectrum: $\gamma_{max.}^{CHCl_3}$, no OH absorption. 1710 (O-benzoyl) and 1685 cm^{-1} (ketone). Nuclear magnetic resonance spectrum: τ 4.75 (1H, multiplet, $w_{1/2}$ 15 cps), 6.25 (1H, quartet, splitting 16 and 7.5 cps); 6.1 (1H, quartet, splitting 16 and 8 cps); 7.2 (1H, quartet, splitting 16 and 2 cps) 8.1 (1H, quartet, splitting 16 and 4 cps), 9.05 (3H, doublet, splitting 4.5 cps); aromatic signal, 5H, between τ 2 - 2.5.

ANHYDROALOPECURINE

Alopecurine (25 mg) was dissolved in benzene (10 ml) and 0.2 ml of thionyl chloride was added. The solution was heated under reflux for one hour on the steam-bath and then kept overnight at room temperature. Part of the solvent was removed at the pump, water added and the solution made

basic with ammonium hydroxide. The benzene layer was separated and the aqueous layer was extracted several times with fresh portions of benzene. The benzene extracts were combined and evaporated. The residue was made up of at least three components (t.l.c.). The main component, anhydrolycocernuine, was obtained in pure form by subjecting the mixture to chromatography on alumina, anhydrolycocernuine being eluted with a mixture of benzene-ether (1:1).

Infrared spectrum: $\gamma_{max.}^{CHCl_3}$, no OH absorption. 1715 (O-benzoyl) 1590, 1605 cm^{-1} (aromatic). Nuclear magnetic resonance spectrum τ 4.7 (1H, multiplet), 4.5 (1H, doublet, splitting 9 cps), 4.65 (1H, quartet, splitting 9 and 2 cps); 6.25 (1H, quartet, splitting 16 and 6.5 cps) 7.55 (1H, quartet, splitting 17 and 8.5 cps); 7.0 (1H, quartet, splitting 16 and 1 cps), 8.0 (1H, quartet, splitting 17 and 2.5 cps); 9.0 (3H, doublet, splitting 6 cps); 5 aromatic protons, 5H, 2 - 2.5 τ. Molecular weight 349 (mass-spectrometry).

Mass spectrum (percentage of base peak in brackets): 349 (18), 334 (4), 244 (26), 227 (86), 205 (91), 201 (52), 170 (100), 144 (34), 122 (34), 105 (19), 91 (37), 77 (79).

SODIUM BOROHYDRIDE REDUCTION OF DEHYDROALOPECURINE

Dehydroalopecurine (6 mg) was dissolved in methanol and sodium borohydride (10 mg) added. The solution was left at room temperature for ten hours. When worked up in the usual manner, the only product isolated from the reaction was alopecurine (t.l.c.).

ACETYLATION OF ALOPECURIDINE

Alopecuridine (55 mg) was dissolved in acetic anhydride-pyridine (6 ml of 1:1) and heated on the steam bath for twenty four hours (the same result was obtained at room temperature). The solvent was removed at the pump and the residue dissolved in chloroform and washed with bicarbonate solution, dilute hydrochloric acid, and water. Evaporation of the chloroform left a solid which was crystallized from acetone to give colorless, fine needless (50 mg), m.p. 223 - 224°.

Analysis. Calculated for $C_{18}H_{27}O_4N$: C, 67.26; H, 8.47; N, 4.36%. Found: C, 67.01; H, 8.75; N, 4.18%. Molecular weight 321 (mass spectrometry). Infrared spectrum: $\gamma_{max.}^{Nujol}$ 1740, 1685 and 1620 cm^{-1}; OH absorption (see Fig. 6). Nuclear magnetic resonance spectrum: τ 7.9 (3H, singlet), 8.9 (3H, doublet, splitting 5 cps).

O-ACETYLHEXAHYDROALOPECURINE

Alopecurine (25 mg) was dissolved in methanol (20 ml) and platinum oxide (10 mg) was added. The solution was subjected to hydrogenation for twenty four hours at room temperature and 50 psi. The catalyst was removed by filtration and the solvent evaporated. The residue obtained in this way was acetylated following the standard procedures. Work up in the usual manner led to the isolation of O-acetylhexahydroalopecurine.

Molecular weight, 415 (mass spectrometry), corresponding to $C_{25}H_{37}O_4N$. Ultraviolet spectrum: Devoid of any characteristic absorption.

ALOPECURINE METHIODIDE

Alopecurine (6 mg) was dissolved in acetone (6 ml), a few drops of methyl iodide added and the mixture warmed on the steam bath for two hours. The solvent was evaporated at the pump, leaving a glassy residue. When this residue was dissolved in a few drops of acetone-ether (3:1), the methiodide, a white powder, separated on scratching.

Alopecurine methiodide, m.p. 250°. Infrared spectrum: $\gamma_{max.}^{Nujol}$ 3360, 1730, 1605, 1590 cm^{-1}.

ACETYLATION OF "BASE 16"

"Base 16" (15 mg) was dissolved in a mixture of pyridine-acetic anhydride (1:1)(2 ml) and the solution left for twenty four hours. The solvent was removed at the pump and the residue dissolved in chloroform and washed with distilled water. Evaporation of the chloroform left a product consisting of two components of similar R_F value. The spectroscopic characteristics of this mixture have been discussed in the text.

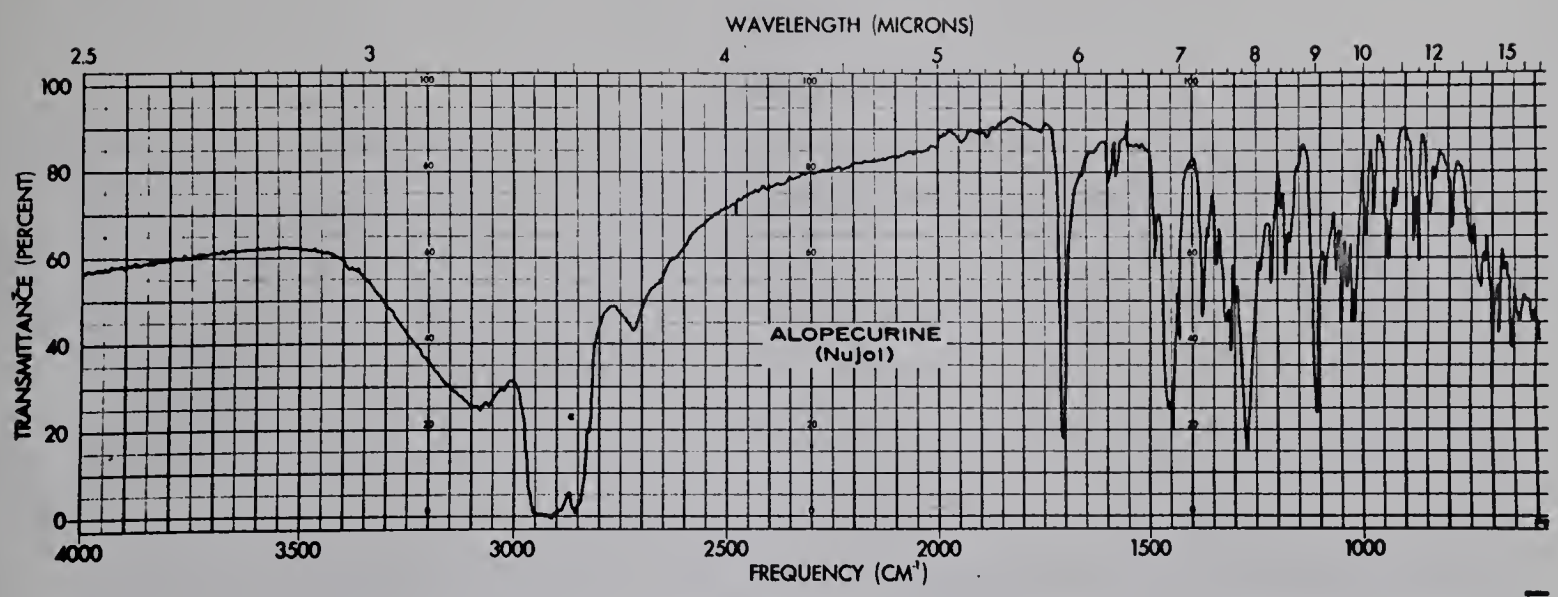
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
ALOPECURINE
(Nujol)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM⁻¹)

FIG. 1

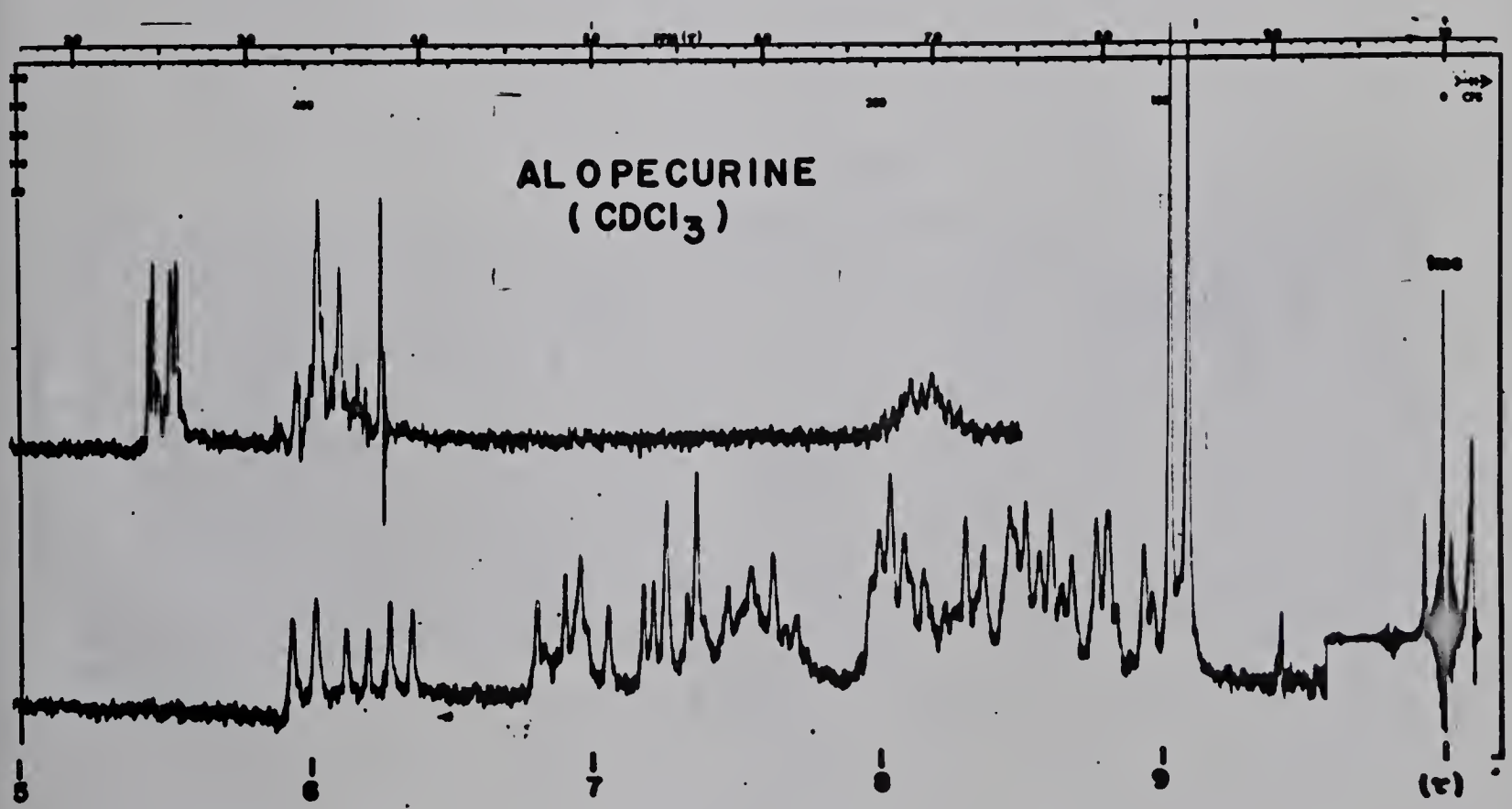
ALOPECURINE
(CDCl3)
5
6
7
8
9
(τ)

FIG. 2

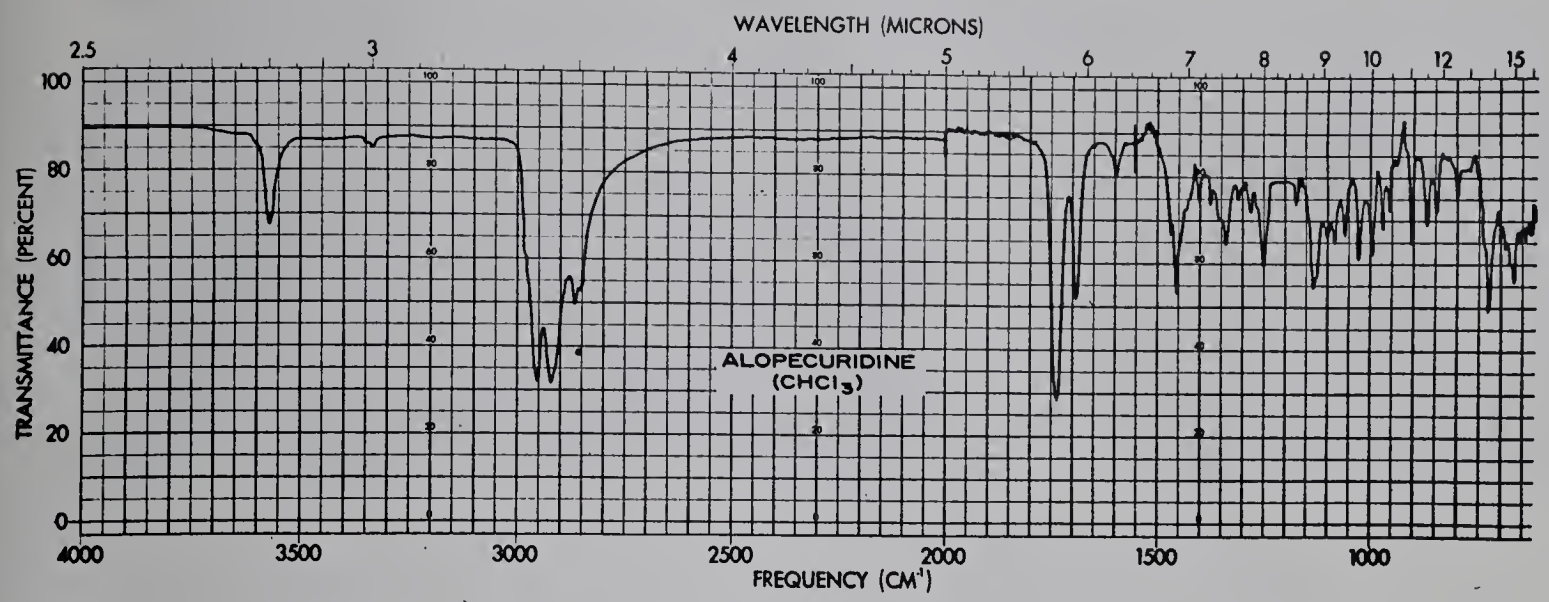
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
ALOPECURIDINE
(CHCl3)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM-1)

FIG. 3

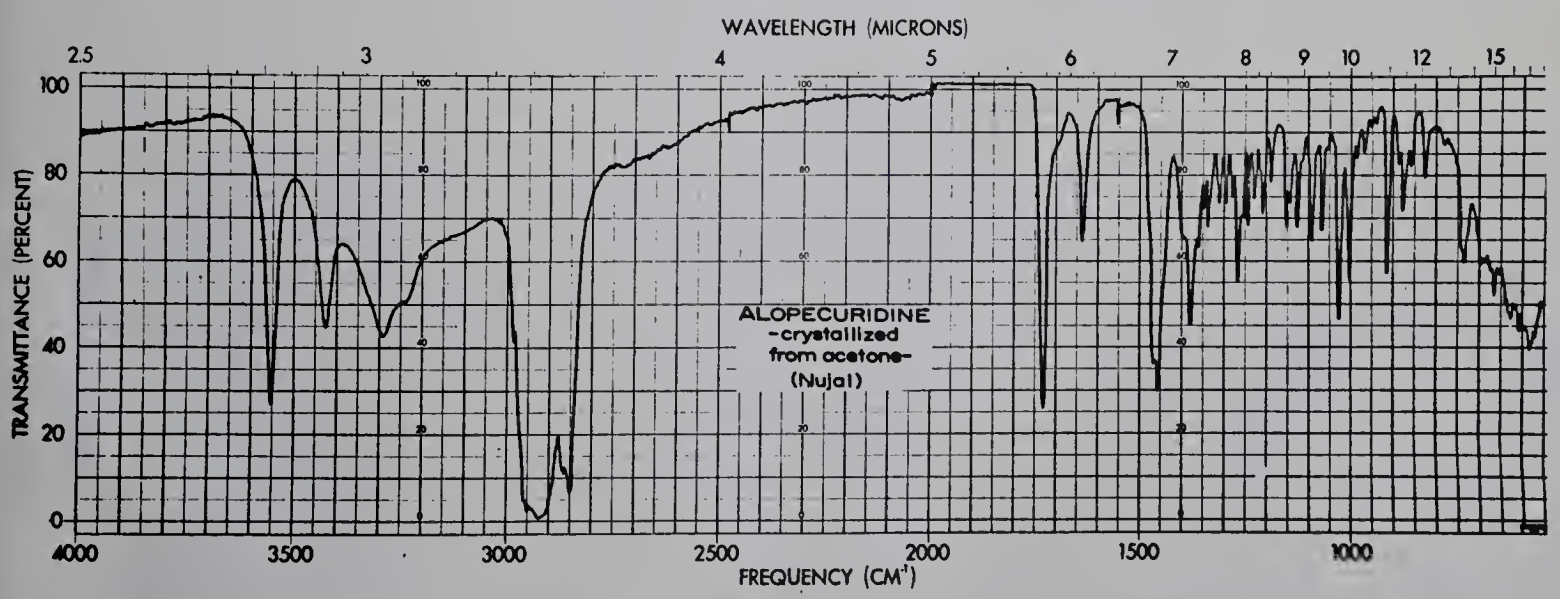
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
ALOPECURIDINE
-crystallized
from acetone-
(Nujol)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM-1)

FIG. 4

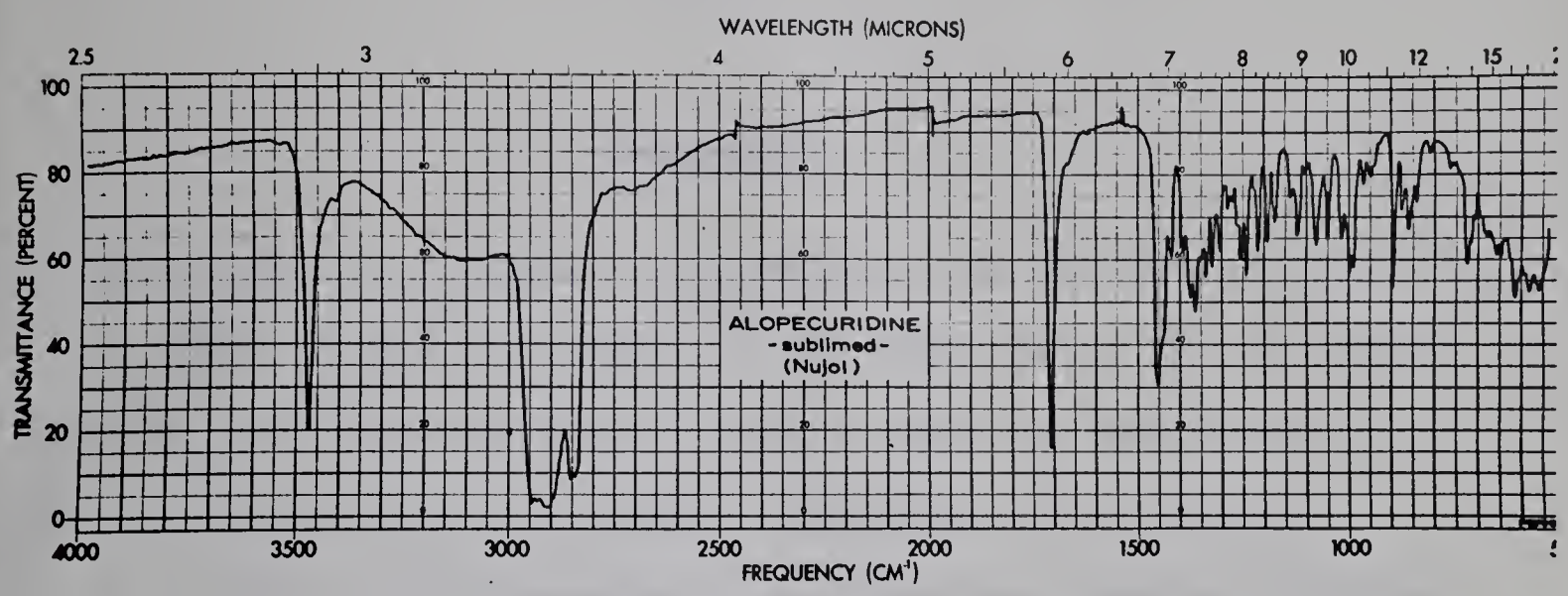
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
ALOPECURIDINE
-sublimed-
(Nujol)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM⁻¹)

FIG. 5

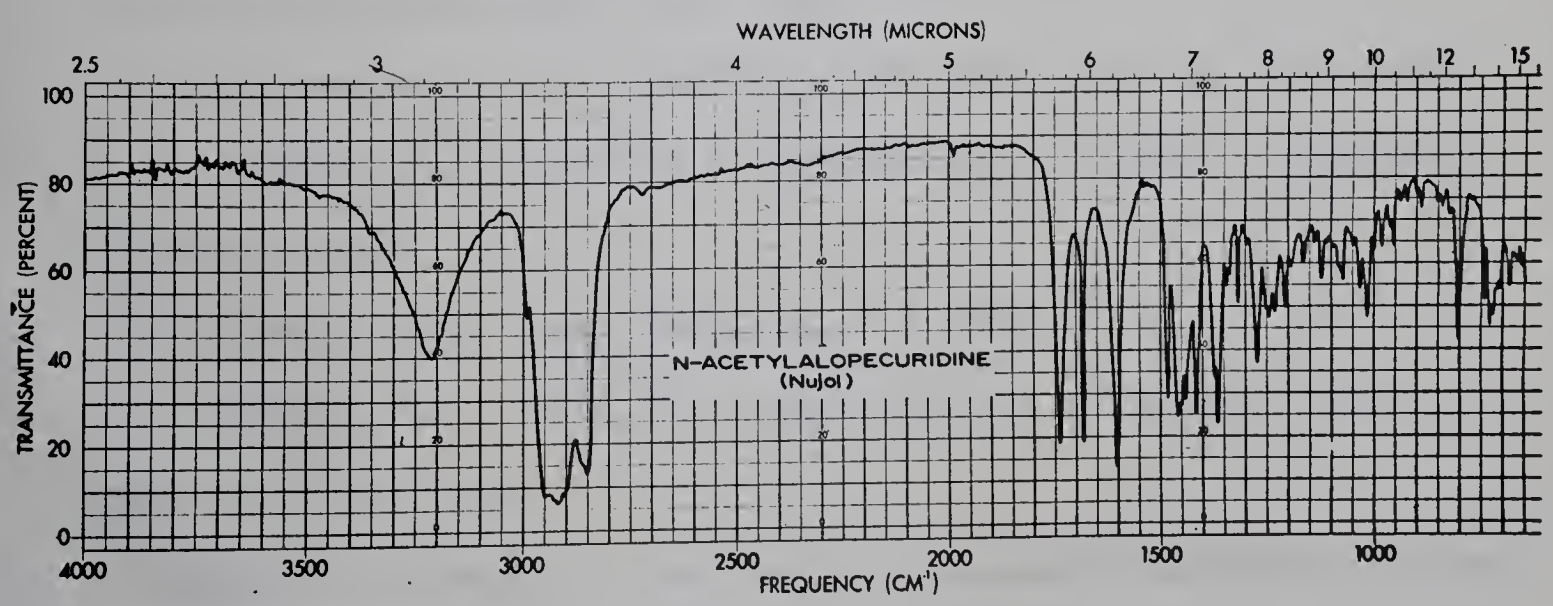
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
TRANSMITTANCE (PERCENT)
100
80
60
40
20
0
N-ACETYLALOPECURIDINE
(Nujol)
4000
3500
3000
2500
2000
1500
1000
FREQUENCY (CM⁻¹)

FIG. 6

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